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Research Article

ANTI STRESS ACTIVITY OF *MURRAYA KOENIGII*; IN RAT MODEL OF ACUTE AND CHRONIC STRESS

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ABSTRACT

Stress basically is a reaction of mind and body against change in the homeostasis. During stressful conditions, the energy requirements of the organism are increased, resulting in enhanced generation of free radicals. The present study was designed to evaluate the potential usefulness of leaf extract of *Murraya koenigii (MK)* for its antistress and adaptogenic activity against oxidative stress induced changes in rats**.** *Withania sominifera (WS),* an adaptogen was used as reference standard. Wistar Albino rats (180-200 g) were used in the study. Animals were divided into 6 groups of six each and treated with vehicle or MK extract (100 and 200 mg/kg, p.o), or WS extract (200 mg/kg, p.o). Except vehicle control group, all groups were subjected to Ischemic/reperfusion induced myocardial injury (IRMI)/ Chronic cold restraint stress (CRS)/ Iron overload induced hepatotoxicity (IO). At the end of the experimental period all rats were sacrificed and heart, liver tissues were collected for biochemical estimations. In IRMI, there was significant reduction in superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) activities and increase in lipid peroxidation LPO activity indicating the production of oxidative stress. In CRS model, stimulation of hypothalamus pituitary adrenal axis (HPA) alters plasma glucose, triglyceride, cholesterol and corticosterone levels. In IO the hepatic LPO activity was significantly increased. Pretreatment with leaf extract of *MK* reverted above stress induced enzyme levels. The results indicating that leaf extract of *MK* has antistress and adaptogenic activity against a variety of biochemical and physiological perturbations in different stress models.

KEY WORDS

Murraya koenigii, Oxidative stress, Antioxidant enzymes, Lipid peroxidation.

INTRODUCTION

In this modern era, stress has become an integral part of human life [1]. Stress is characterized by an imbalance between body demands and the capacity of the body to cope with them. The productive stress is called Eustress while harmful stress is called Distress. If the stress level increases beyond the threshold limit of an individual, it results in decreased performance and stress-induced disorders. Under these conditions, stress triggers a wide range of body changes called General Adaptive Syndrome (GAS). The stimuli which produce GAS are called the stressors and range from physical to psychological factor including cold, heat,

infection, toxins, and major personal disappointment etc [2].

During stressful conditions, the energy requirement of the organism is increased, resulting in enhanced generation of free radicals [3]. During this process, the ability of the body's defensive system to combat the oxidative stress may diminish due to reduced antioxidants. It is possible to support the body's adaptation by using food supplements, dietary elements, herbs and minerals for increasing physical and mental performance. In the indigenous system of the medicine, there are many herbal drugs and formulations recommended to enable one to withstand stress without altering the physiological functions of the body. This drug induced state of resistance against aversive stimuli is termed as Adaptogenic activity and the drugs are named as Adaptogens [4]. Many herbs reported in ancient literature have potent anti stress activity and their utilities in current scenario need to be unveiled.

Murraya koenigii Linn (MK) is an aromatic plant that is widely cultivated for its aromatic leaves [5]. Almost every part of this plant has a strong characteristic odour. The people of this plain, particularly of southern India, use the leaves of this plant as a spice different curry preparations [6]. The plant has been used in traditional Indian medicine for a range of ailments. The whole plant is considered to be a tonic and stomachic. Roots and Barks are stimulants and are applied externally for skin eruptions and poisonous bites. Green leaves are Febrifuge and used in Dysentery [5, 6].

It contains carbazole alkaloids namely murrayanine [7], mahanimbine [8], girinimbine [9], murrayacine [10], mahanine, koenine, koenigine, koenidine, koenimbine [11] etc. The leaves are fair sources of vit A and are also rich source of calcium, phosphorous, iron, thiamine, riboflavin, niacin, vit C, carotene etc. The major constituents of the curry leaf are monoterpenes (70%) seed cotyledons (86%), constituting pinene (52%) and cis ß ocimene(45%). The essential oil from *murraya koenigii* leaves was reported to have antibacterial [12], antifungal [13], and the ethanolic extract of curry leaf has shown antiprotozoal [14], hypoglycemic activities [15]. In another study antioxidant activity of *murraya koenigii* leaves against DPPH radicals and nitric oxide levels has been reported [16, 17]. Khan et al., [18], reported the hypolipedemic activity of curry leaf in albino rats. Since *murraya koenigii* has a number of medicinal properties including free radical scavenging activity, the present study as under taken to evaluate the potential usefulness of fresh leaves of *murraya koenigii* for its Antistress and Adaptogenic Activity against oxidative stress induced changes in experimental Animals. *Withania somnifera*, an established Ayurvedic herb used as an Adaptogen, was used as reference standard [19].

MATERILAS AND METHODS

Preparation of the plant extract:

Fresh leaves of *murraya koenigii* (5kg) were collected locally; the leaves were cleaned and dried in shade. The dried leaves were crushed in to a coarse powder and were defatted with petroleum ether, then it was extracted with 95% ethanol using soxholet apparatus for 8 Hrs and the resultant extract was evaporated using rotary vacuum evaporator and it was kept in vacuum desiccators to obtain fine powder. The yield obtained was 12%.

Animals: Wistar Albino rats of either sex (180-200 gm) were used. The rats were fed with standard pellet diet (Ratan brothers, Hyd) and water *ad libitum*. They were housed on polypropylene cages maintained under standard conditions (12hr: 12hr light dark

cycle, 25±3°C, 36-60% RH). The experiments protocols were subjected to the scrutinisation of the institutional Animal ethics committee and were cleared by the same principles of laboratory animal care (NIH publication No 82-23, revised 1985) guidelines were followed.

Animal treatment schedule: Animals were divided into 6 groups of six each and group1 served as vehicle control and group 2 was subjected to IRI/CRS/IO and group 3 received 200mg/kg of MK only without any stress regimen. Group 4, 5 received 100 &200 mg/kg of MK along with any of the stress regimen. Group 6 received 200mg/kg of WS with stress regimen, considered as standard.

Ischemia – Reperfusion myocardial injury (IRMI):

The isolated rat heart perfusion technique [20] was used in brief. Rats were anesthetized with pentobarbitone sodium (40mg/kg i.p) and the hearts were dissected out. The aorta was perfused with Krebs-heinslet solution at 37°c on a Langen drof's apparatus. The perfusion cycle used to induce IRMI was a slight modification of the method described earlier [20]. The 30 min perfusion cycle consisted of normal perfusion for 5min, followed by stoppage of the perfusion (Ischemia) for 10min, and finally restoration of perfusion (Reperfusion) for 15min. Control rat hearts were per fused for 30 min continuously. At the end of each cycle the heart was removed, weighed and processed for the Biochemical estimations.

MK (100 and 200mg/kg, P.O), WS (200 mg/kg, P.O) and the vehicle were administered for 5 days, the last administration being 1hr prior to the perfusion experiments. The rats were divided in to 6 groups of 6 each, one group served as normal control received only vehicle, second group served as an IRI group with vehicle only. Third group received 200mg/kg of MK with normal perfusion. Fourth and fifth group received 100 and 200 mg/kg, P.O of this MK along with IRI respectively. Sixth group received WS (200mg/kg, P.O) along with IRI.

Chronic cold restraint stress:

The drugs were administered orally 45min prior to the stress regimen up to 7 consecutive days except that the rats were kept fasted overnight on the sixth day after drug feeding and stress exposure. The stress was produced by restraining the Naïve animals inside an adjustable cylindrical plastic tube (6.2 cm diameter 20 cm long). The rats were confined individually and exposed continuously to cold stress at 4±1 °c for 50 min once only for 7 consecutive days [21]. On day seven rats were sacrificed immediately after stress regimen and blood was collected for estimation of various biochemical parameters such as plasma glucose, cholesterol, triglycerides and serum corticosterone. Similarly the weights of organs, i.e. liver and adrenal glands were also recorded.

Iron overload induced Hepatotoxicity:

Iron overload was induced by administration of ferrous sulfate (30mg/kg, i.p) [22 . Vehicle, MK, WS were administered for 5 days once daily one hour prior to iron over load. On day 5th the rats were sacrificed 1 hr later, the liver was removed, washed with saline perfusate, weighed and processed for estimation of LPO.

Biochemical estimation:

The tissues was obtained from the different experimental groups were homogenized in 2ml of ice cold triple distilled water and sonicated for 16 sec. thereafter the homogenates were centrifuged (10,000*g for 2min) and the supernatants were used for the estimation of SOD , CAT, GPX. For estimation of LPO activity, the tissues were homogenized in gold potassium chloride (0.5M). The following Biochemical estimations were done.

SOD activity:

Assay was based on the ability of SOD to inhibit the spontaneous oxidation [23] of adrenaline to adenochrome. Results were expressed as units (U) of SOD activity/mg protein. One unit of SOD induced approximately 50% inhibition of auto oxidation of adrenaline.

CAT activity:

The assay was based on the ability of CAT to induce the disappearance of hydrogen peroxide [24] (H_2O_2), which was followed by spectrophotometrically. One unit (U) of CAT was defined as the amount of the enzyme required to decompose 1µM of H_2O_2 /min, at 25°c and pH 7.0. Results were expressed as units (U) of CAT activity /mg protein.

GPX activity:

H_2O_2 was used as the substrate. Sodium azide (1mM) was added to the reaction mixture [25] to inhibit the remnant CAT activity. One unit of GPX was defined as the amount of enzyme decomposing 1M H_2O_2 /min at 25°c and pH 7.0. Results were expressed as units (U) of GPX activity /mg protein.

LPO activity:

LPO was determined by estimating the accumulation of peroxidate product, thiobarbituric acid reactive substance [26] (TBARS), using a standard curve of 1, 1, 3, 3-tetra methoxy propane and was expressed as n mol TBARS/ gm tissue.

Protein estimation:

Protein was estimated was by the method of Lowry et al [27] method.

Statistical analysis:

All the results were expressed as mean ±SEM and analyzed by one way analysis of variance (ANOVA) followed by Dunnete's multiple comparison test. P value < 0.05 was considered as being statistically significant.

RESULTS

Ischemic reperfusion myocardial injury:

In IRMI there was significant ($p<0.05$) reduction in the levels of SOD, CAT, GPX and significant increase in LPO indicating the production of oxidative stress. The results were summarized in table 1. MK (100, 200 mg/kg, P.O) and WS (200 mg/kg, P.O) administered once daily for 5 days tended to increase cardiac SOD, CAT, GPX concentrations with concomitant decrease in LPO. However the results remains statistically insignificant in normally (30 min) per fused hearts.5 days pre treatment with MK and WS significantly reverse the IRMI induced decrease in SOD, CAT, GPX activity respectively.

Table .1 Effect of ethanolic extract of *M. koenigii* on SOD, CAT, GPX and LPO in isolated heart model of IRI

Treatment(mg/kg P.O) n=6	SOD U/mg protein	CAT U/mg protein	GPX U/mg protein	LPO nmol TBARS/g
Vehicle +normal perfusion NP(30min)	29.48±3.56	44.63±3.95	6.94±0.12	52.4±3.48
Vehicle +IRI	13.65±1.84[a]	15.35±2.42[a]	2.32±0.04[a]	93.62±5.42[a]
WS 200mg/kg +IRI	26.32±2.72[b]	39.48±2.92[b]	4.62±0.08[b]	62.43±3.56[b]
MK 100mg/kg +IRI	18.58±2.89[b]	23.78±2.36[b]	3.92±0.06[b]	72.46±4.25[b]
MK 200mg/kg +IRI	27.46±3.04[b]	41.36±3.56[b]	5.43±0.09[b]	48.35±3.42[b]

Data is represented as mean ±SEM (n=6). Vehicle and test drugs were administered once daily for 5 days prior to the perfusion experiments.

a. P<0.01 compared to the normal perfusion group (NP)
b. P<0.05 compared to the Ischemia reperfusion (IRI) group

Chronic CRS:

In Chronic cold restraint stress pretreatment with MK and WS were 7 days prior to stress exposure, offered significant ($p<0.05$) protection against the changes in the weights of liver, spleen and adrenal gland when compared to stress control. The extract dose dependently decreased the elevated levels of biochemical parameters ($p<0.05$). The results were given in **Table 2 and 3**.

Table.2 Effect of ethanol extract of *M. koenigii* on biochemical parameters in cold restraint stress

Treatment(mg/kg P.O) n=6	Corticosterone µg/dl	Glucose mg/dl	Cholesterol mg/dl	Triglyceride mg/dl
Vehicle only	95.35±2.96	90.46±0.42	91.62±1.56	69.46±2.57
Chronic CRS	164.98±2.82 [a]	184.29±1.56 [a]	198.64±3.48 [a]	99.78±1.85 [a]
WS 200mg/kg +CRS	109.48±2.48 [b]	98.60±1.85 [b]	95.04±1.89 [b]	63.22±2.95 [b]
MK 100mg/kg +CRS	139.48±3.46 [b]	136.96±3.39 [b]	146.03±3.02 [b]	88.46±1.56 [b]
MK 200mg/kg +CRS	115.58±3.35 [b]	120.49±2.43 [b]	98.60±1.62 [b]	79.56±1.89 [b]

Data is represented as mean ±SEM (n=6). Vehicle and CRS test drugs were administered once daily for 7 days 45 min prior to the chronic CRS.

a. P<0.01 compared to the normal control
b. P<0.05 compared to CRS

Table. 3. Effect of ethanol extract of *M.koenigii* on organ weights in cold restraint stress

Treatment (mg/kg P.O) n=6	Liver (g/100g)	Adrenal gland (mg/100g)
Vehicle only	3.56±1.58	8.96±0.43
Chronic CRS	5.96±1.97 [a]	15.42±0.98 [a]
WS 200mg/kg +CRS	3.71±1.23 [b]	9.46±0.57 [b]
MK 100mg/kg +CRS	5.04±2.58 [b]	12.43±1.23 [b]
MK 200mg/kg +CRS	4.12±1.69 [b]	10.09±1.09 [b]

Data is represented as mean ±SEM (n=6). Vehicle and CRS test drugs were administered once daily for 7 days 45 min prior to the chronic CRS.

a. P<0.01 compared to the normal control
b. P<0.05 compared to CRS

Iron over load induced hepatotoxicity:

Pretreatment with *MK* (100 and 200 mg/kg, P.O) and WS (200 mg/kg P.O) once daily for 5 days tended to decrease hepatic LPO activity and the effects were statistically significant ($p<0.01$). The results were given in **Table 4**.

Table .4 Effect of ethanolic extract of *M.koenigii* on LPO in iron overload induced hepatotoxicity (IO) in rats.

Treatments (mg/kg P.O) n=6	Lipid peroxidation nmol TBARS/g
Vehicle only	23.76±1.68
Iron overload (IO)	62.47±3.23 [a]
WS 200mg/kg+IO	27.63±1.84 [b]
MK 100mg/kg+IO	42.78±2.93 [b]
MK 200mg/kg+IO	25.43±1.73 [b]

Data is represented as mean ±SEM (n=6). Vehicle and CRS test drugs were administered once daily for 7 days 45 min prior to the chronic CRS.

a. P<0.01 compared to the normal control
b. P<0.01 compared to CRS

DISCUSSION

Stress represents the reaction of the body to stimuli that disturb its normal physiological equilibrium or homeostasis, often with detrimental effects [28]. The human society has become complex and, in many ways, more demanding. The failure of successful adaptation during stressful situations has resulted in stress-related illnesses that result from, or are associated with dysregulation of the stress response [29].

Adaptogens [30] are the plant derived biologically active substances, which appear to induce a state of non-specific increase of resistance of the organism to diverse aversive assaults which threaten internal homeostasis and which improve physical endurance for doing work even in adverse circumstances and in difficult environmental conditions. Stress involves complex biochemical, neurological and immunological mechanisms and places a crucial role in the genesis or progression of a variety of disease states such as psychiatric disorder like depression, anxiety, immunosuppression, endocrine disorder including Diabetes mellitus, impotency and cognitive dysfunction.

A variety of stress situations have been employed to investigate the consequences of stress and to evaluate anti stress agents and the lack of consistency of stress protocols and their biological consequences is astounding. In Ischemic reperfusion induced myocardial injury after a period of flow reduction, there is an increased cardiac lipid peroxidation products, and decreased SOD, CAT and GPX activities [32]. Pretreatment with *MK* extract produces significant decrease in lipid peroxidation activity and SOD, CAT and GPX activities are restored suggesting that *MK* having adaptogenic activity.

Exposure of the animals to the Cold restraint stress caused severe imbalance and resulted in adrenal hypertrophy, indicating the active involvement of the hypothalamic -pitutary-adrenal (HPA) axis, which is highly responsive to stress. Adrenal hypertrophy takes place in response to the secretion of adrenocorticotrophic hormone (ACTH) from the pituitary due to increased Corticosterone levels from cortical cells to combat stress in the normal physiological conditions [31]. In the present study there was a significant increase in blood glucose level was observed because adrenal cortex secretes Cortisol in man and Corticosterone in rats under stressful conditions. Internal homeostasis is maintained through the process of gluconeogenesis and lipogenesis by hyper secretion of cortisol (Krupavaram et al., 2007). In stress- induced animals there was

marked increase in serum cholesterol, triglycerides and corticosterone due to stimulation of HPA and sympathetic system, resulting in production of catecholamines and glucocorticosteroids [34]. Administration of MK and WS extracts significantly ($p<0.05$) decreased the elevated levels of serum cholesterol, triglycerides, plasma glucose and Corticosterone.

Under chronic stressful conditions there is increased production of corticotropic hormone that leads to increase in weight of adrenals [33]. *M. koenigii* and *W.somnifera* extract administration significantly reduced the liver and adrenal gland weight this may be due to decreased production of corticotropic hormone by reversing the stress-induced adrenomedullary response.

Iron overload believed to cause organ damages through generation of reactive oxygen species, ROS. The release of ROS is one of the major signaling pathways involved in many different forms of apoptotic cell death in many cell types [32]. Released ROS leads to the increased production of Lipid peroxidation products. Pretreatment with *MK* and *WS* extract prior to the stress exposure results in decreased Lipid peroxidation activity.

Pre treatment with *MK* leaf extract significantly attenuated all the stress induced changes confirming its anti stress activity. Further the extract offered a significant protective effect against perturbations in the levels of antioxidant enzymes with marked decrease in LPO, suggesting a decrease in oxidative damage. Earlier studies reveal that *MK* contains carbazole alkaloids namely murrayanine, mahanimbine, girinimbine, murrayacine, mahanine, koenine, koenigine, koenidine, koenimbine etc. which were reported to possess antioxidant activity. Thus, the strong antioxidant potential of *MK* might be responsible for its antistress activity, since many natural plant products possess antistress activity due to their antioxidant activity.

CONCLUSION

The present study provides conclusive evidence for the antistress activity of *Murraya koenigii* against different models of oxidative stress with diverse etiologies. However clinical investigations are required to confirm these observations.

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