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IN-VIVO ANTI-OXIDANT ACTIVITY OF ETHANOLIC EXTRACT OF *HUGONIA MYSTAX* LINN LEAVES IN STREPTOZOTOCIN-NICOTINAMIDE INDUCED DIABETIC RATS

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ABSTRACT

To evaluate the *In-vivo* anti-oxidant potential of ethanolic extract of *Hugonia mystax* leaves in Streptozotocin - nicotinamide induced diabetic rats. The present studies revealed that *Hugonia mystax* has significant *in-vivo* antioxidant activity and can be used to protect tissue from oxidative stress. The result showed that the activities of glutathione, SOD, catalase and peroxidase in group treated with Glibenclamide declined significantly than that of normal group. Ethanolic extract of *Hugonia mystax* in the dose of 400 mg/kg, p.o., has improved the glutathione, SOD, catalase, and peroxidase levels significantly, which were comparable with Glibenclamide. Based on this study we conclude that Ethanolic extract of *Hugonia mystax* possesses *in vivo* antioxidant activity and can be employed in protecting tissue from oxidative stress.

KEYWORDS

Hugonia mystax, *In-vivo* anti-oxidant potential, Ethanolic extract, Streptozotocin-nicotinamide and Wistar Albino Rats.

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INTRODUCTION

The genus *Hugonia* L. of family Linaceae comprise about 40 species in the world. This plant *Hugonia mystax* is locally known as Modirakanni. Ethnobotanically, the fruits are used by the tribals of Kalakad Mundanthurai for the treatment of Rheumatism. Roots were used as anthelmintic, astringent and also used for dysentery, snake bite, fever, inflammation and rheumatism. Biological activities such as analgesic, anti-inflammatory and ulcerogenic were also reported. Roots of *Hugonia*

mystax were evaluated for preliminary phytochemical screening and antimicrobial activity. Preliminary phytochemical screening showed the presence of various classes of secondary metabolites such as flavonoids, phenols, saponins, steroids, tannins and terpenoids. Antimicrobial activity of petroleum ether, chloroform, ethanol and aqueous extracts of root extracts showed significant activity against various human pathogens.

Taking into consideration of medicinal value and utility, the present study was planned to explore the *In-vivo* anti-oxidant potential of the medicinal plant named *H. mystax.*

MATERIALS AND METHODS

Fresh leaves of *H. mystax* were collected from velliangiri hills from Coimbatore. Leaves of *Hugonia mystax* was dried in shade for two weeks. Dried leaves were coarsely powdered, sieved (#40) and stored in an air tight container at room temperature. Dried powder was then extracted sequentially with petroleum ether, chloroform, and ethanol using soxhlation method. The extracts were concentrated to dryness using rotary evaporator. The yields of various extracts were found to be 4.5% w/w (petroleum ether), 4.7% w/w (chloroform) and 10.5 % w/w (ethanol). All the extracts were preserved in a refrigerator at 4 ^{o}C. Also phytochemical analysis and antidiabetic study were done in the ethanolic extract.

Wistar Albino Rats (Male) weighing around 150-200 gm was selected for the experiment. All the animals were randomly divided into five groups with six animals each. The extract and standard group animals were treated once a day for 14 days as follows. The first group received normal saline (control), the second group received STZ + Nicotinamide induced rats (diabetic control), the third group received ethanolic extract of *Hugonia mystax* leaves (200mg/kg, orally), the fourth group received ethanolic extract of *Hugonia mystax* leaves (400mg/kg orally), The fifth group received Diabetic Control + Standard (Glibenclamide 5mg/kg orally). On day 14 the rats were sacrificed by carotid bleeding and liver was rapidly excised, rinsed in ice-cold saline, and a 10% w/v homogenate was prepared using 0.15M KCI, centrifuged at 800 rpm for 10 min at 4 ^{o}C. The supernatant obtained was used for the estimation of catalase, peroxidase, and other enzymes. Further, the homogenate was centrifuged at 1000 rpm for 20 min at 4 and the supernatant was used for biochemical estimation of protein content, malondialdehyde (MDA), Lipid hydroperoxides (LH) and anti-oxidant biochemical parameters. (Estimation of glutathione, SOD, catalase, Peroxidase).

RESULTS AND DISCUSSION

Table No.1: Effect of *Hugonia mystax* leaves on liver protein, MDA and LH in the experimental groups

S.No	Group	Dose	Protein	MDA	LH
1	Normal control	1 ml/kg Normal saline	1642±87	182±12	17.42±1.1
2	Diabetic Control	(STZ 60 mg/kg + Nicotinamide 110mg/kg	674±72^{a}	291±26^{b}	22.68±1.9^{b}
3	Positive control	Glibenclamide 5 mg/kg	1458±101^{c}	229±18^{c}	19.2±1.6^{c}
4	EEHM	200 mg/kg	1255±110^{c}	256±17^{c}	21.36±1.2^{c}
5	EEHM	400 mg/kg	1392±106^{c}	248±19^{c}	20.2±1.2^{c}

Values are mean± SEM; n=6. ^{a}P<0.05, ^{b}P<0.01 when compared to control. ^{c}P<0.01, when compared to Glibenclamide control (one way ANOVA followed by Dunnett's test). Protein=n moles/min/mg protein, MDA = n moles/min/mg protein, LH= n moles/min/mg protein.

Table No.2: The antioxidant activity of *Hugonia mystax* leaves in the experimental animals

S.No	Group	SOD	CAT	GSH	Px
1	Control (Normal saline 1ml/kg)	7.81± 0.32	2.01 ±0.49	98.16 ±0.91	3.26± 0.26
2	Diabetic Control (STZ 60 mg/kg + Nicotinamide 110mg/kg	4.36± 0.40[a]	0.76± 0.37[a]	57.32 ±0.88[a]	4.56 ±0.32[a]
3	Glibenclamide 5 mg/kg	6.94± 0.56[b]	1.87± 0.48[b]	89.64± 0.72[b]	2.94± 0.21[b]
4	EEHM 200 mg/kg	7.48± 0.42[b]	1.99± 0.49[b]	96.42 ±0.63[b]	3.18 ±0.28[b]
5	EEHM 400 mg/kg	7.12 ±0.38[b]	1.90 ±0.43[b]	90.06 ±0.72[b]	2.98± 0.19[b]

Values are mean± SEM; n=6. [a]P<0.01 when compared to control [b]P<0.01 when compared to Glibenclamide control (one way ANOVA followed by Dunnett's test) CAT= µmoles/min/mg protein, GSH= n moles/min/mg protein, SOD= n moles/min/mg protein, GSH=n moles/min/mg protein and peroxidase= n moles/min/mg protein.

CONCULSION

This investigation revealed that the *Hugonia mystax* Linn contain pharmacologically active substance (s) such as carbohydrates, flavonoids, steroids, saponins, terpenoids compounds, which are responsible for the antioxidant activity.

Ethanolic extract of *Hugonia mystax* in the dose of 400 mg/kg, p.o. has improved the glutathione, SOD, catalase, and peroxidase levels significantly, which were comparable with Glibenclamide. Based on this study we conclude that Ethanolic extract of *Hugonia mystax* possesses *in-vivo* antioxidant activity and may be employed in protecting tissue from oxidative stress. Literature reveals that, the carbonyl groups present in the flavonoids and phenolic compounds were responsible for antioxidant activity. In future it shows good free radical scavenger and to Antioxidant treatment which can prevent oxidative free radicals. So, it is necessary to have a detailed study on the same.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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