Preliminary phytochemical and toxicity studies of *Oenothera biennis*

***S Lakshman, B Asha Jyothi, V Mounica, M Shasidhar, D Rahamthulla, A Ravi Kumar**
Department of Pharmacology Bapatla College of Pharmacy Bapatla 522101 Andhra India
***Corresponding Author: E-Mail: lakshman.s93@gmail.com**

ABSTRACT

The present investigation was carried out to evaluate the safety of methanol extract from leaves of *Oenothera biennis* by determining its potential toxicity after acute and sub acute administration in mice. For the acute study, *Oenothera biennis* was administrated to mice in single doses given by oral route. General behavior adverse effects and mortality were determined up to7 days. In the Sub acute study, the extract was administered orally at doses of 200 and 400 mg/kg for 28 days to mice Biochemical and hematological parameters were determined at the end of 28 days of daily administration. The studies on sub acute toxicity reveals that no mortalities or evidence of adverse effects have been observed in mice following acute oral administration at the highest dose of 2000mg/kg crude extracts of *Oenothera biennis*. In sub acute toxicity study daily oral administration of methanol extract 200 and 400 mg/kg body weight of *Oenothera biennis* for up to 28 days did not result in death or significant changes in body weight, Hematological and Biochemical parameters were done and tabulated. Preliminary photochemical studies of extracts of *Oenothera biennis* screening of chemical constituents for the presence of Carbohydrates Glycosides Alkaloids Sterols Triterpenoids Proteins Amino Acids Tannins Flavanoids and Fixed Oils were tested.

Key words: *Oenothera biennis*, Toxicity studies, Preliminary screening, Chemical constituents

INTRODUCTION

Nature has best owned upon us a very prosperous botanical prosperity and a large number of diverse types of plants cultivate wild in different parts of our country. In India, the use of different parts of plant *Oenothera biennis.* Experimental screening method is important in order to ascertain the safety and efficacy of traditional and herbal products and also to establish the active component of the herbal products. Therefore, the purpose of this study was designed to determine the acute and sub acute oral toxicity of the leaf extract of *Oenothera biennis*. It contains the pain relieving compound phenylalanine and is increasingly being used to treat chronic headaches. It is currently being studied all over the world as a treatment for aging problems, alcoholism, acne, heart disease, hyperactivity in children, multiple sclerosis, weight control, obesity, PMS and schizophrenia. It has so many preventive and therapeutic qualities that it has become a standard part of recommendations of many herbalists for maintaining youth and preventing disease. Evening Primrose Oil contains a high concentration of a fatty acid called GLA and it is this fatty acid that is largely responsible for the remarkable healing properties of the plant.

In fact, Evening Primrose contains one of the highest concentrations known of this important substance and only a few other plants contain it at all. This makes Evening Primrose an important medicinal herb, and as studies continue, the list of benefits will likely become much longer. The gamma-linoleic acid, linolenic acid and other nutrients in this oil are essential for cell structure and improve the elasticity of the skin. Prevent diabetes-associated nerve damage Research indicates that the GLA in evening primrose oil can help prevent, and in some cases even reverse, the nerve damage (neuropathy) so commonly seen with diabetes. In a year-long study, such symptoms as numbness, tingling, and loss of sensation in participants with mild diabetic neuropathy were less marked in those who took evening primrose oil than in those who took a placebo. Reduce the symptoms of eczema in some cases, eczema develops when the body has problems converting dietary fats into GLA. Getting supplemental GLA from evening primrose oil may therefore be helpful. Some studies indicate that this oil can outperform a placebo in relieving eczema-related inflammation, as well as the itching, oozing, and flaking associated with this condition. By taking GLA, eczema sufferers may tolerate reduced doses of steroid creams and drugs, many of which cause unpleasant side effects Combat damage from multiple sclerosis the abundant supply of essential fatty acids in evening primrose oil may be valuable in minimizing the inflammation associated with this progressive nerve disorder.

The fatty acids may also contribute to healthy nerve development when taken over time. Treat Alzheimer's-related memory deficiencies by boosting the transmission of nerve impulses, evening primrose oil may be valuable in treating this progressive brain disorder. Counter impotence and female infertility by promoting blood flow, the GLA in evening primrose

oil can help treat a primary cause of male impotence; compromised circulation leading to impaired penile blood flow.

MATERIALS AND METHODS

Plant Material Collection: The leaves of *Oenothera biennis* were collected from coastal Andhra. It was authenticated by Prof. Dr. A. Ravi Kumar Department of Pharmacognosy, Bapatla college of Pharmacy, Bapatla Guntur District, Andhra Pradesh, India.

Table.1.Phytochemical screening of Methanolic extract of *Oenothera biennis*

Phytoconstituents	Extract
Carbohydrates	+
Glycosides	+
Alkaloid	+
Sterols	+
Triterpenoids	+
Proteins & Amino acids	-
Tannins	+
Flavonoids	+
Fixed oils	+

+ present; -absent

Experimental animals: Healthy mice weighing 20-35 gm were acclimatized for 14 days. The animals were housed under standard conditions and room temperature (25±2oC). During the acclimatization period of 14 days, animals were observed for general condition every day and weighed on the next day of arrival and on the last day of acclimatization. The experimental protocol was approved by the Institutional Animal Ethical Committee of Committee.

Acute toxicity study: The toxicity study as carried out using mice (20-35 g).The acute toxicity studies were conducted as per the OECD guidelines 420(OECD 2000) where the limit test dose of 2000 mg/kg was used. The animals were divided into one control group and one treated group, each group consisting of ten animals (10 animals). Behavioral signs like behavior were observed.

Sub acute-Toxicity Study: Healthy adult mice weighing 20-30 gm were divided in to 3 groups of 6 animals each and were housed under standard conditions and room temperature (25±2oC). The control animals (Group-I) received 0.5ml of vehicle alone and the other two groups(Group-II &III) have received Oenothera biennis extract for 28 days at doses of 200,400 mg/Kg body weight respectively.

Observations: Toxic manifestations and mortality were monitored daily and body wt changes were recorded every 7 days till the end of the study.

Hematological Biochemical Studies: At 28thday animals were fasted for 12 hrs, they anaesthetized with ether and blood was collected from orbital sinus for the analysis of hematological parameters using Mythic18, which included Hemoglobin, Red blood cell count, white blood cell count, platelet, reticulocyte, neutrophils, Eosinophils, lymphocytes, monocytes, packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin concentration, mean corpuscular hemoglobin and was centrifuged at 4000 rpm at 4o C for 10 minutes to obtain the serum for biochemical estimations. Both the plasma and serum were stored at -20o C until analyzed for biochemical parameters. The serum was assayed for bilirubin, serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, serum alkaline phosphatase, serum proteins, serum total albumin, serum total globulin, serum cholesterol, serum triglycerides, creatinine, blood urea nitrogen, calcium, phosphorus and electrolytes like sodium, potassium and chloride using auto analyzer. Immediately after collecting the blood samples, animals were then sacrificed by ether anesthesia.

Statistical analysis: All the results are expressed as mean value ± Standard deviation (S.D).

RESULTS AND DISCUSSION

Acute Toxicity Study: The acute toxicity study was conducted as per the OECD guidelines 420, where the limit test dose of 2000mg/Kg was used. The observations are presented in Table .No test substance related mortality was observed at 2000mg/Kg and throughout the observation period there were no significant changes in the body weight and treatment related change like respiration rate and heart rate. Persistent treatment related changes were observed in behavioral signs viz apathy, reduced locomotor behavior but regained after 24 hrs. Consequently, 2000 mg/Kg of plant extract found safe with less toxic effect.

Sub acute toxicity study: The methanolic extract of *Oenothera biennis* at dose of 200,400 mg/kg orally for every 24 hr for 28 days did not produce any mortality in tested animals. No sign of observable toxicity was detected during the experimental period. Progressive increase in body weight at dose of 200,400 mg/kg of mice during 28 days of administration of methanolic extract of *Oenothera biennis* may indicate the improvement in the nutritional state of the animal.

Table.2.Observations of Acute Toxicity of *Oenothera biennis*

Animal no	Dose mg/ Kg	Body wt.(gm)	Apathy	Ataxia	Circling	Compulsive behavior	Excitability	Locomotor behaviour	Moribund	Drinking	Edema	Paralysis	Reflexes	Heart rate	Respiratory rate	Pruritis	Eyelid closure	Diarrhea	Depression	Body wt. changes	Hunched/stiff posture
A1	200	31	+	-	-	-	-	+	-	-	-	-	-	N	N	-	N	-	-	-	-
A2	200	30	+	-	-	-	-	+	-	-	-	-	-	N	N	-	N	-	-	-	-
A3	200	35	+	-	-	-	-	+	-	-	-	-	-	N	N	-	N	-	-	-	-
A4	200	30	+	-	-	-	-	+	-	-	-	-	-	N	N	-	N	-	-	-	-
A5	200	32	+	-	-	-	-	+	-	-	-	-	-	N	N	-	N	-	-	-	-
A6	200	30	+	-	-	-	-	+	-	-	-	-	-	N	N	-	N	-	-	-	-
A7	200	30	+	-	-	-	-	+	-	-	-	-	-	N	N	-	N	-	-	-	-
A8	200	30	+	-	-	-	-	+	-	-	-	-	-	N	N	-	N	-	-	-	-
A9	200	30	+	-	-	-	-	+	-	-	-	-	-	N	N	-	N	-	-	-	-
A1	200	30	+	-	-	-	-	+	-	-	-	-	-	N	N	-	N	-	-	-	-
C1	C	30	N	N	N	N	N	N	N	N	-	-	N	N	N	N	N	-	-	N	N
C2	C	30	N	N	N	N	N	N	N	N	-	-	N	N	N	N	N	-	-	N	N
C3	C	35	N	N	N	N	N	N	N	N	-	-	N	N	N	N	N	-	-	N	N
C4	C	30	N	N	N	N	N	N	N	N	-	-	N	N	N	N	N	-	-	N	N
C5	C	30	N	N	N	N	N	N	N	N	-	-	N	N	N	N	N	-	-	N	N
C6	C	30	N	N	N	N	N	N	N	N	-	-	N	N	N	N	N	-	-	N	N
C7	C	35	N	N	N	N	N	N	N	N	-	-	N	N	N	N	N	-	-	N	N
C8	C	30	N	N	N	N	N	N	N	N	-	-	N	N	N	N	N	-	-	N	N
C9	C	30	N	N	N	N	N	N	N	N	-	-	N	N	N	N	N	-	-	N	N
C1	C	30	N	N	N	N	N	N	N	N	-	-	N	N	N	N	N	-	-	N	N

+ Significant changes; - not observed/no change noticed; C-Control;N- normal

Hematological and Biochemical parameters: The effect of *Oenothera biennis* extract on hematological parameters of the experimental and control mice is presented in table 4. All the tested hematological parameters such as hemoglobin, R.B.C, Platelet count, Reticulocyte count, Mean corpuscular volume, mean corpuscular hemoglobin concentration, Percent of Neutrophils, Lymphocytes and Monocytes, Packed cell volume and mean corpuscular hemoglobin remained within physiological range throughout the treatment period (28 days).

The data for the biochemical parameters in the treated and control mice are presented in Table Sub acute oral administration of *Oenothera biennis* extract (daily for 28 days) did not cause any significant changes in some biochemical parameters including serum bilirubin, Serum total proteins, serum total albumin, serum total globulin, serum cholesterol, serum triglyceride, sodium, potassium, calcium and phosphorus and the activity of the marker enzymes of the liver (Serum glutamic oxaloacetic Transaminase, Serum Glutamic pyruvic Transaminase, Serum alkaline phosphatase)

Herbal medicine is still the mainstay of about 75-80% of the world population, mainly in the developing countries for primary health care. Herbal medicines have received greater attention as an alternative to clinical therapy and the demand for these remedies has currently increased. The increase in number of users as oppose to the scarcity of scientific evidences on the safety of the medicinal plants have risen regarding toxicity and detrimental effects of these remedies. The medicinal plants commonly contain various bioactive principles which have the potential to cause beneficial and/or detrimental effects. Experimental screening method is important in order to ascertain the safety and efficacy of traditional and herbal products and also to establish the active component of the herbal products. The results of the acute toxicity reveals that there was no mortality observed up to the maximum dose level of 2000mg/kg b.wt of the extract administered orally, which is the single high dose recommended by OECD guidelines423 for testing acute toxicity. No changes attributable to treatment were found in body weight, respiration rate, heart rate. Treatment related changes observed in behavioral signs viz. apathy, reduced locomotor behavior but regained after 24 hr may be due to the effect of solvent.

Thus the present investigation reveals that methanolic extract of *Oenothera biennis* does not cause any acute toxicity. Generally the reduction in body weight gain and internal organ weights is a simple and sensitive index of toxicity after exposure to toxic substances. In sub-acute toxicity study mice treated with 200,400 mg/kg doses of methanolic

extract of *Oenothera biennis* had a progressive increase in body weight. The increase in weight was not significantly different from that of the control. The progressive increase in body weight at dose of 200,400 mg/kg of mice during 28 days of administration of methanolic extract of *Oenothera biennis* may indicate the improvement in the nutritional state of the animal. The growth response effect could be as a result of increased food and water intake. The hematopoietic system is one of the most sensitive targets for toxic chemicals and an important index of physiological and pathological status in human and animal the hematological status after 28 days of oral administration of methanolic extract of *Oenothera biennis* was also assessed. The white blood cell was found to be significantly increased in Group – II and decreased in Group-III. With the exception of a transient change in WBC count there were no significant alterations in the hematological parameters. Increase in WBC may indicate the impact of *Oenothera biennis* in boosting the immune system of treated groups. However slight changes in WBC did not show any dose responsiveness. All the other hematological parameters in all treated group remained normal without any significant difference.

Transaminases (GOT and GPT) and ALPs are good indices of liver damage. There were no deleterious changes found in the level of transaminases and ALPs in serum of treated groups with control animals. Equally, there also was no marked change in creatinine in these two doses when compared to the control. And creatinine is known as an effective indicator of renal function and any rise in creatinine levels is observed if there is marked damage to functional nephrons. Thus, the results recorded in this study suggest that *Oenothera biennis* extract did not affect the renal function. Clearly, this only serves as a preliminary test and that for a better estimation of renal function a creatinine clearance test is required. The liver is the site of cholesterol disposal or degradation and the major site of synthesis. Since, no significant changes were observed in cholesterol levels in this study, it suggests that *Oenothera biennis* extract had no effects on the cholesterol metabolism of the mice. All other biochemical parameters such as total protein, albumin and globulin were remained normal without any significant difference. The levels of electrolytes maintain the body fluid equilibrium. No significant changes were observed in the electrolytes levels, except Calcium, Chloride and blood urea nitrogen. Calcium, Chloride and blood urea nitrogen were significantly changed in treated animals when compared with control group suggesting that *Oenothera biennis* extract was relatively low or non-toxic under study conditions.

Table.3. Hematological parameters after 28 days oral treatment with methanol extract values are expressed as mean ± S.D of *Oenothera biennis*

Parameters	Group-I	Group-II	Group-III
Hemoglobin G%	15.48±0.53	15.45±0.67	15.93±0.51
RBC X 106/cmm	8.46±0.30	8.48±0.36	8.77±0.23
WBC X 103/ cmm	4.07±0.41	5.32±0.47	3.98±0.81
PLT lakhs/cmm	5.72±0.61	6.3±0.45	6.35±0.36
PLT lakhs/cmm	0.97±0.13	1.02±0.18	1±0.18
Neutrophil %	20.5±3.94	21.67±2.84	24±7.56
Lymphocyte%	78.17±4.06	77.17±5.62	74.83±7.53
Monocyte %	1.33±0.46	1.17±0.36	1.17±0.36
PCV%	45.82±1.12	45.33±2.16	47.15±1.41
MCV FI	54.24±1.67	54.49±2.13	53.77±1.21
MCH pg	18.28±0.50	18.2±0.81	18.1±0.48
MCHC gm/dl	33.77±0.37	34.05±0.59	33.75±0.39

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Table.4.Effect of treatment with extract on biochemical parameters Values are expressed as S.E.M of *Oenothera biennis*

Parameter		Group-I	Group-II	Group-III
SGOT IU/L		123.17±22.61	115.83±22.51	
SGPT IU/L		80.83±11.55	77.66±13.62	90.5±13.92
ALP IU/L		581.66±86.21	539.83±47.34	500.83±128.41
BILI mg/dl		0.43±0.093	0.42±0.10	0.5±0.07
PRO g/dl		5.1±0.48	5.12±0.43	4.97±0.44
ALB g/dl		2.33±0.11	2.4±0.12	2.33±0.13
GLB g/dl		2.75±0.21	2.61±0.21	2.87±0.22
Cholesterol mg/dl		82.83±4.15	82.67±5.98	82.67±4.31
TG mg/dl		90.67±3.58	94.17±4.40	93.83±8.73
Electrolytes	**Na mEq/L**	150.48±7.10	158.68±2.10	150.12±10.36
	K mEq/L	6.8±1.01	6.78±0.57	5.96±0.72
	Cl mEq/L	116.82±6.96	130.45±6.93	115.47±8.07
	Ca mg/dl	8.5±0.32	8.53±0.37	9.88±0.81
	P mg/dl	7.03±0.47	6.77±0.61	7.18±0.31
BUN mg/dl		18.35±8.50	9.15±0.52	11.3±2.91
Creatinine mg/dl		0.33±0.05	0.25±0.07	0.32±0.12

CONCLUSION

In conclusion, this study provides the very valuable data on the acute and sub acute toxicity profile of the methanol extract of *Oenothera biennis* that should be very useful for any future in vivo and clinical study of this plant medicine. *Oenothera biennis* extract was found to be less toxic when oral acute and sub acute toxicities in mice were performed. Chronic toxicity, are necessary to further support the safe use of this herb. These results showed that the use of extract of *Oenothera biennis* is safe and explained the extensive utilization of the plant in traditional medicine. Preliminary chemical constituents of methonolic extract of chemical constituents of *Oenothera biennis* were screened.

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