Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 6, 2015

Original Article

ACUTE AND SUB-ACUTE TOXICITY STUDY OF AQUEOUS METHANOLIC LEAF AND BARK EXTRACT OF *DOLICHANDRONE ATROVIRENS*

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Received: 02 Mar 2015 Revised and Accepted: 28 Mar 2015

ABSTRACT

Objective: The present study was to evaluate the oral toxicity of acute and sub acute studies of methanol leaf and bark extract of *Dolichandrone atrovirens*

Methods: In acute toxicity studies of aqueous methanolic *Dolichandrone atrovirens* leaf extract(DALE) and *Dolichandrone atrovirens* bark extracts (DABE) (in 0.3 % sodium CMC) as a single dose (2000 mg/kg) was administered to the Swiss albino mice (20-25 g) by oral route and the animals were observed for mortality and any toxic symptoms up to 14 days. In sub acute toxicity studies the DALE and DABE were administered daily for 28 days at doses ranging from 200-400 mg/kg. The animals were found in signs of toxicity, morbidity and mortality for 28 days. The animals were submitted to observe the serum biochemical markers and weight of the vital organs.

Results: The results of 14 days acute toxicity studies up to a dose of 2000 mg/kg of the aqueous methanolic DABE and DALE neither produced mortality nor shows any symptoms of behavior or any physiological changes in body weight, food and water intake. 28 days sub acute studies repeated doses of oral toxicity did not show any toxic signs or any mortality when three doses 200 and 400 mg/kg of the methanolic leaf and bark extracts of *Dolichandrone atrovirens* administered. No significant changes were integrated in biochemical and hematological parameters when compared with the control group.

Conclusion: From the results it is concluded that the dose at 400 mg/kg is safe for long term treatment in diabetic conditions.

Keywords: Aqueous methanolic, *Dolichandrone atrovirens* leaf extract (DALE), *Dolichandrone atrovirens* bark extracts (DABE), Acute Toxicity, Sub acute toxicity.

INTRODUCTION

The pharmacological treatment of disease began with the use of herbs [1]. Worldwide the recognition of herbal medicine was increased. In recent years, an increasing use of herbal medicines in developed countries has expanded sharply [2]. The World Health Organization (WHO) estimates that 80% of the world's population used the plant-based medicines as their primary medical intervention [3, 4]. At the same time medicinal herbs produced some notable toxicity like renal and hepatic toxicity [5]. In India, the use of herbal medicine is the Vedic period and the popular medical plants which are reported in texts like Ayurveda and Sidha. Most of the new drugs which are approved from 1994 are based on natural products [6]. The use of herbal drugs for the management of certain ailments continues unabated in most developing communities due to easy access and for economic reasons [7]. In the absence of reliable liver-protective drugs in allopathic medical practices, herbs play a role in the management of various liver disorders [8].

Dolichandrone atrovirens are used in Indian traditional system to treat rheumatism, arthritis, diabetes, inflammation, and liver diseases. Diuretic and antispasmodic effects are reported for the use of *Dolichandrone atrovirens* seeds. The powder is applied externally to treat swelling and decoction of the bark is provided for stomach pain [9, 10] and also has the antioxidant and antidiabetic activity [11, 12]. There are a lot of herbals was used to treat diabetics, but the toxicity has not been properly addressed. The present study was conducted the toxicity of the aqueous methanol leaf and bark extract of *Dolichandrone atrovirensactive*. Hence, the leaf and bark extract was submitted to acute and sub acute toxicity studies to further confirm these activities using animals.

MATERIALS AND METHODS

Plant material

The leaves and bark of *Dolichandrone atrovirens* were collected from Chitheri hills, Salem in the month of November 2009. The plant was then authenticated and a voucher specimen is related for further reference.

Preparation of extract

The shade dried coarse powders of the plant material (1.5 kg) were extracted with 80 %v/v aqueous methanol by maceration at room temperature for 72 h and the extract was filtered, concentrated to dryness in a rotavapor under reduced pressure and controlled temperature (40-50 °C). The extractive values and nature of the leaf and bark extracts of *Dolichandrone atrovirens* were administered orally to the experiments.

Experimental animals

Healthy Swiss albino mice and wistar rats were fed with standard animal pellet diet and water ad libitum. The experiment protocols received clearance from the Institutional Animal Ethical Committee (IAEC) and CPCSEA, Chennai, India.

Acute toxicity

The overnight fasted Swiss albino mice (20-25 g) were weighed and administered the methanolic leaf and bark extracts of *Dolichandrone atrovirens* (in 0.3 % sodium CMC) as a single dose (2000 mg/kg) by oral route and the animals were observed for toxic symptoms continuously for first 4 hours.

Number of survivors was noted after 24 hours and the observation made daily for a period of 14 days. Toxic symptoms for which the animals were found in 72 hrs included behavioral changes, convulsions, locomotion and mortality. Cage side observations included changes in skin, fur, eyes, mucous membranes, somato-motor activity, respiratory, autonomic, central nervous systems, and behavior pattern. Special attention was addressed to observations of tremors, lethargy, sleep convulsions, salivation, diarrhea, and coma. Body weight, water and food intake were accounted for.

Subacute toxicity

Five groups of adult male albino rats (150-160g) were used for sub acute toxicity. Group 1 (control treated with 0.3% sodium CMC (2 ml/kg, p. o), Group 2 (methanolic leaf extract of D. atrovirens 200 mg/kg, p. o), Group 3-Methanolic leaf extract of D. atrovirens 400 mg/kg, p. o), Group 4 (methanolic bark extract of D. atrovirens 200 mg/kg, p. o) and Group 5 (methanolic bark extract of D. atrovirens 400 mg/kg, p. o)

After completion of the study period, blood samples were harvested from overnight fasted animals after 24 h of the last dose of plant extracts and analyzed the hematological parameter. In addition, blood glucose, total cholesterol, serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), gamma glutamate transpeptidase (GGTP), Alkaline phosphatase (ALP), BUN, total bilirubin, urea, total protein, albumin and creatinine were also estimated. The urine analysis was performed to investigate any abnormalities in the excretion pattern after the exposure with a test drug for 28 days. The urine was harvested from each animal at the end of the study early in the morning and it was analyzed for the detection of abnormal constitutions.

RESULTS

Acute toxicity

Methanolic lead and bark extracts of D. atrovirens did not cause any mortality in mice up to 2000 mg/kg. None of the doses tested produced any apparent gross effect on general motor activity, fecal output, muscular weakness, feeding behaviour etc. during the period of observation. This indicated that the extracts were considered to be safe at the tested dose level.

Sub-acute toxicity

During the experimental period of 28 days, the increase in body weight in all test groups was not significantly different from control group. At the end of the study period, no statistically significant differences were seen in the mean haemoglobin content. WBC, RBC, hematocrit, clotting time and differential cell counts of all test groups when compared to control group no and mean biochemical parameters. The consequence of urine analysis does not show any abnormalities in the excretion pattern.

DISCUSSION

The safe dose range of drug can be determining by the LD_{50} values through acute toxicity studies [13, 14]. In the present study, animals treated with DALE and DABE did not show any toxic symptoms or mortality when dosed up to 2000 mg/kg body weight by oral route. This indicates that the extracts are found to be harmless at the tested dose level. Sub-acute toxicity study gives valuable information on the cumulative toxicity of a substance on target organs or physiological and metabolic effects of the compound at a low dose on prolonged exposure. A wide variety of adverse effects can be detected with sub acute toxicity studies. The result from such studies can give information, which will aid in selecting dose level. The long term safety level of a compound can be expected from acute or shorter than sub acute studies [15, 16].

Table 1: Effect of DALE and DABE on body weight of the extract treated animals

Design of treatment	Day 0	Day 7	Day 14	Day 21	Day 28
Control	150.5±3.61	154.7±3.72	160.7±2.71	167.5±4.03*	175.3±3.28*
DALE 200	151.4±3.54	155.5±2.41	162.8±2.31*	166.7±2.38**	172.4±1.23***
DALE 400	153.7±1.69	156±2.26	160.8±2.41	167.7±3.11***	176.6±3.24***
DABE 200	154.5±1.69	158.17±1.83*	163.3±1.55***	168.3±1.41***	173.5±2.12***
DABE 400	153±1.74	157.7±1.76	162.3±2.44**	169.1±1.92***	174±2.86***

N=6; Values were expressed as mean±SEM; Data were analyzed by one way ANOVA followed by Tukey Kramer multiple comparison test; NS-Not significant when compared to control; *P<0.05, **P<0.01, ***P<0.001 when compared to Day '0'.

Table 2: Effect of DALE and DABE on hematological parameters of the extract treated animals

Parameters	Control	DALE 200	DALE 400	DABE 200	DABE 400
HGB (g/dL)	15.32±0.15	14.98±0.52	15.9±0.26	15.93±0.17	14.2±1.13
RBC (M/µL)	5.05±0.05	5.53±0.31	5.35±0.13	5.4±0.09	5.26±0.39
HCT (%)	46.17±0.65	41.95±2.6	47.83±1.01	48±0.68	41.17±3.53
PLT (K/µL)	485±16.68	558±53.34	575±36.58	613±28.59	637±64.40
WBC (K/µL)	7.13±0.65	8.68±0.94	7.85±0.66	9.83±0.40	9.76±2.65
LYM (%)	82.3±1.75	84.5±1.08	84.5±2.01	82.6±1.43	72.8±6.75
MID (%)	12.18 1.11	11.15±0.64	10.83±1.39	12±0.93	15.27±2.39
GRANULO (%)	5.52±0.76	4.32±0.65	4.63±0.86	5.41±0.87	7.42±0.24

N=6; Values were expressed as mean±SEM; Data were analyzed by one way ANOVA followed by Tukey Kramer multiple comparison test; NS-Not significant when compared to control.

Table 3: Effect of DALE and DABE on serum hepatic and renal parameters of extract treated animals

Parameters	Control	DALE 200	DALE 400	DABE 200	DABE 400
SGOT (IU/l)	188±4.15	194.5±4.96	198.33±5.14	201±6.34	193.16±6.06
SGPT (IU/l)	80.17±2.65	90.73±3.68	94.33±2.45	93.7±4.0	94.83±4.64
ALP (IU/l)	191±6.66	214.67±9.6	219±6.52	221.67±6.37	223.3±11.24
GGTP (IU/l)	43.83±2.21	45±2.32	45.5±2.52	44±1.84	48.67±0.76
Total Bilirubin (mg/dL)	0.6±0.05	0.65±0.07	0.633±0.07	0.583±0.06	0.633±0.07
Glucose (mg/dL)	84.9±0.95	85.97±1.10	85.63±0.85	83.03±0.76	85.9±1.09
Total cholesterol (mg/dL)	102±8.30	102.8±6.74	111.5±10.57	114.3±6.28	100.9±7.29
Urea (mg/dL)	57.5±3.14	53.3±2.33	62.67±3.13	58±5.02	62.5±5.49
BUN (mg/dL)	26.78±1.48	24.88±1.09	29.23±1.48	27.1±2.33	29.17±2.57
Creatinine (mg/dL)	0.75±0.02	0.733±0.03	0.817±0.03	0.8±0.02	0.817±0.03
Total Protein (g/dL)	6.92±0.09	7.1±0.06	7.13±0.04	6.9±0.05	6.83±0.14
Albumin (g/dL)	4.067±0.06	4.183±0.07	4.217±0.07	4.083±0.07	4.00±0.11

N=6; Values were expressed as mean±SEM; Data were analyzed by one way ANOVA followed by Tukey Kramer multiple comparison test; NS-Not significant when compared to control.

Table 4: Urine analysis of DALE and DABE treated animals

Parameters	Control	DALE 200	DALE 400	DABE 200	DABE 400
Appearance	Straw Yellow	Straw Yellow	Straw Yellow	Straw Yellow	Straw Yellow
Specific gravity	1.02±0.003	1.03±0.0034	1.04±0.003	1.02±0.004	1.02±0.002
pH	6.42±0.03	6.43±0.02	6.47±0.02	6.47±0.02	6.43±0.02
Glucose	NIL	NIL	NIL	NIL	NIL
Proteins	NIL	NIL	NIL	NIL	NIL
Ketone bodies	NIL	NIL	NIL	NIL	NIL
Blood cells	NIL	NIL	NIL	NIL	NIL

N=6; Values were expressed as mean±SEM; Data were analyzed by one way ANOVA followed by Tukey Kramer multiple comparison test; NS-Not significant when compared to control.

Table 5: Effect of DALE and DABE on weight of vital organs of extract treated animals

Name of organs	Weight of organs (g/100g of body weight)				
	Normal	DALE 200	DALE 400	DABE 200	DABE 400
Liver	4.34±0.09	4.86±0.09	4.72±0.13	4.62±0.2	4.68±0.18
Kidneys	1.03±0.05	1.18±0.04	1.10±0.04	1.16±0.04	1.21±0.05
Heart	0.63±0.01	0.64±0.02	0.62±0.03	0.59±0.01	0.63±0.01
Lungs	1.04±0.01	1.17±0.04	1.17±0.07	1.06±0.03	1.01±0.06
Spleen	0.34±0.02	0.39±0.01	0.41±0.02	0.38±0.02	0.39±0.02

N=6; Values were expressed as mean±SEM; Data were analyzed by one way ANOVA followed by Tukey Kramer multiple comparison test; NS-Not significant when compared to control.

End of the study period, no statically significant differences were seen in the mean Hb content, WBC, RBC and differential cell counts of all test groups when compared with control group. Further there were no changes observed in the serum biochemical markers *viz*., SGOT, SGPT, ALP, Total protein, Total cholesterol, Total bilirubin level and clotting time in all test groups when compared to control group. The consequence of urine analysis does not show any abnormalities in excretion pattern.

Both the leaves and bark extract of *Dolichandrone atrovirens* are free from adverse effects on hepatic and renal systems and their function. There was not any mortality for administered leaves and bark extract of *Dolichandrone atrovirens* at a very high dose. The weight of the principal organs also showed that, there were no statistically significant differences observed in all test groups when compared to control group.

CONCLUSION

The results of the present study concluded that aqueous methanol leaf and bark extract of *Dolichandrone atrovirens* was non toxic and no marked changes in hematological, biochemical parameters. However, further studies have to be order to evaluate long term toxicities.

CONFLICT OF INTERESTS

Declared None

REFERENCES

1. Schulz V, Hänsel R, Tyler VE. Rational Phytotherapy. A Physician's Guide to Herbal Medicine. 4th Ed. Berlin, Springer-Verlag; 2001. p. 306.
2. Blumenthal M, Busse WR, Goldberg A, Gruenwald J, Hall T, Riggins CW, *et al*. The complete german commission e monographs: therapeutic guide to herbal medicines, Austin, TX/Boston, MA, American Botanical Council/Integrative Medicine Communications; 1998. p. 318.
3. Ogbonnia S, Adekunle AA, Bosa MK, Enwuru VN. Evaluation of acute and subacute toxicity of Alstonia congensis Engler (Apocynaceae) bark and Xylopia aethiopica(Dunal) A. Rich (Annonaceae) fruits mixtures used in the treatment of diabetes. Afr J Biotechnol 2008;7:701-5.
4. Rickert K, Martinez RR, Martinez TT. Pharmacist knowledge of common herbal Preparations. Proc West Pharmacol Soc 1999;42:1-2.
5. Jaouad EH, Israilli ZH, Lyoussi B. Acute toxicity and chronic toxicological studies of Ajuga iva in experimental animals. J Ethnopharm 2004;91:43–50.
6. Butler MS. Natural products to drugs: natural product-derived compounds in clinical trials, Nat Prod Rep 2008;25:475.
7. Renjit Bino Kingsley, Saminathan, Pemaiah Brindha, Appian Subramoniam. Toxicity studies of the active fraction of *Stereospermum tetragonam* DC. Int J Pharm Pharm Sci 2013;5(3):648-51.
8. Sivakrishnai SA, Kottaimuthu A. Hepatoprotective activity of ethanolic extract of aerial parts of *Albizia procera roxb* (*benth.*) against paracetamol induced liver toxicity on wistar rats. Int J Pharm Pharm Sci 2014;6(1):233-8.
9. Nadkarni AK. In: Indian Materia Medica, Popular Prakashan Pvt Ltd, Bombay. Vol. I. 1996. p. 680-3.
10. Kirtikar KR, Basu BD. Indian Medicinal Plants. Vol I. 2nd Edn. International book publisher, Dehradun; 1993. p. 714-6.
11. Kavimani S, Saminathan K, Senthil Kumar R. *In vitro* antidiabetic activity of *Dolichandrone atrovirens*–an Indian medicinal plant. Int J Pharmacother 2014;4(3):107-13.
12. Kavimani S, Saminathan K, Senthil Kumar R. Antioxidant and free radical scavenging activities of *Dolichandrone atrovirens* using various *in vitro* assay models. Int J Phytopharmacol 2014;5(4):293-300.
13. OECD, Organisation for economic cooperation and development. Repeated dose 28-day oral toxicity test method guideline 407 adopted 23.03.1996. In: OECD, Guidelines for testing of chemicals. Paris: Organisation for Economic Co-Operation and Development; 1995.
14. OECD, Organisation for Economic Cooperation and Development. Guidelines for the Testing of Chemicals/Section 4:Health Effects Test No. 423:Acute oral toxicity-acute toxic class method. Paris: Organization for Economic Cooperation and Development; 2002.
15. Narayan KMV, Bowman BA, Engelgau ME. Prevention of type 2 diabetes: New study from Finland shows that lifestyle changes can be made to work. B M J 2001;323:63-4.
16. Goodpaster BH, Katsiara A, Kelley DE. Enhanced fat oxidation through physical activity is associated with improvements in insulin sensitivity in obesity. Diabet 2003;52:2191-7.