IOSR Journal Of Pharmacywww.iosrphr.org
(e)-ISSN: 2250-3013, (p)-ISSN: 2319-4219
Volume 7, Issue 10 Version. 1 (October 2017), PP. 20-25

Subchronic Toxicity Study of Hydroethanolic Extract of *Delphinium Denudatum* Root And *Amaranthus Spinosus* Leaves on Experimental Rats

*Mohd. Abid[1], Tahira Sultan[1], Naman Gupta[1], Alankar Shrivastav[1], Najam Ali Khan[1]

[1]*Department of Pharmacology, School of Pharmaceutical Sciences, IFTM University, Moradabad-244001, U.P., India*

*Corresponding Author:*M Abid[1]*

Abstract:

Object: *The objective of this study is to evaluate subchronic toxicity study of hydroethanolic extract of Delphinium denudatum root and Amaranthus spinosus leaves. Products that obtained from plants have been used for medicinal purposes. Approximately about 80% of the world population today, relies on natural drugs to meet their health needs. The problem with the natural preparations as the drug is that most of the plants are been used indiscriminately without adequate information on associated safety/ toxicity risks. It was thought that natural drugs considered being safe but it is proved to be toxic. Therefore, there is a need to be maintaining the proper knowledge, safety/ toxicity profile and scientific documentation guidance of these natural products.*

Material and methods: *Subchronic toxicity study of hydroethanolic extract of Delphinium denudatum root and Amaranthus spinosus leaves was evaluated by estimation of hematological, biochemical parameters, mean body and organ weight of the animals.*

Results and conclusion: *In the recent study it was found that DDE, ASE and combination of both the drugs extracts showed lesser amount of toxicity compare to control group. However, it could be argued that these changes may not be toxicologically significant and no mortality of animals was observed throughout the study period.*

Keywords: *Toxicity, hematological, Biochemical, Delphinium denudatum, Amaranthus spinosus.*

Date of Submission: 06-10-2017 Date of acceptance: 26-10-2017

I. INTRODUCTION

Products that obtained from plants have been used for medicinal purposes. Approximately about 80% of the world population today, relies on natural drugs to meet their health needs [1]. The problem with the natural preparations as drugs is that most of the plants are been used indiscriminately without adequate information on associated safety/ toxicity risks. It was thought that natural drugs considered being safe but it is proved to be toxic, therefore, there is lack of proper knowledge and guidance of these natural products, therefore there is a need for scientific documentation on the safety/ toxicity profile on these acclaimed medicinal plants [2].

Delphinium denudatum (Jadwar; *Ranunculaceae*) is one of the important medicinal drugs used as indigenous medicine in India; the entire plant is reported to be useful in a variety of ailments [3]. The root is used in various medical formulations in Unani and Ayurveda to reduce the withdrawal symptoms in people on de-addiction therapy [4].

Herbalists recommend the roots in the treatment of fungal infections, dysurea, calculi, asthma, cough, jaundice and nervous problems [5].

Amaranthus spinosus is used as febrifuge, antipyretic, laxative and diuretic. Besides its culinary value, it is a popular medicinal plant used to reputed for treat digestible, bronchitis, leprosy, epilepsy, piles and as a treatment for hallucination, healing of wounds and rheumatism, and to arrest the coughing up of blood. All parts of the plant are known to contain medicinally active constituents [6, 7].

In the traditional system of medicine herbal formulation and combined extracts of plants are used as drug of choice rather than single drug and they show the toxicity either by drug-drug interaction or any type of mechanism. Therefore, recent study was planned to carry out the subchronic toxicity study of *Delphinium denudatum* and *Amaranthus spinosus* singly as well as in combination of both the drugs.

II. MATERIAL AND METHODS

2.1 Collection, Identification and Authentification of drugs:

Plant material like root of *Delphenium denudatum* was collected from local market of Moradabad Uttar Pradesh and leaves of *Amaranthus spinosus* from IFTM university botanical garden. *Amaranthus spinosus* leaves were authenticated by the Botanist, Dr. Beena Kumari, Hindu College, Moradabd. A plant specimen (Voucher No. HC.MBD/HAP/BK/2016/01/488) was submitted in the herbarium and *Delphenium denudatum* root was authenticated by Dr. Ashok Kumar IFTM University, Moradabad Uttarpradesh and plant specimen was kept in the herbarium with the Voucher No. 2015/SOS/BOT/14. The botanical taxonomy of the plants was properly matched with standard floras and also cross-checked with Herbarium files.

2.2 Preparation of extracts

The dried plant materials were powdered and passed through a 20-mesh sieve. The coarsely dried powdered materials of each plant were taken then drugs were defatted with petroleum ether followed by extracted with hydro-alcoholic mixture (Ethanol 95%, v/v: water, 1:1) in a Soxhlet apparatus. The extracts were filtered and concentrated separately by distilling off the solvents and evaporated to dryness using rotatory vaccum evaporator.

2.3 Animals

Experiments were performed on either sex of albino wistar rats weighing (150-200g). Animals were procured from the animal house of the I.F.T.M. University, Moradabad and maintained on a natural day–night cycle (12hr dark: 12hrs light) at room temperature of about 24-26°C, with free access to standard food pellets and water *ad libitum*. Animals were acclimatized for at least ten days before exposure to behavioral experiments. Experiments were carried out between 10:00-17:00 hours. The study was approved by Institute Animal Ethics Committee, Department of Pharmacology and Clinical Research, College of Pharmacy, IFTM University, Moradabad. All experimental were in observance with the Animal Ethical Committee, Committee for the Purpose of Control And Supervision of Experiments on Animals (CPCSEA) and were approved by University Ethical Committee with an approval number 837/Po/Re/S//04/CPCSEA.

2.4 Subchronic toxicity study

Repeated-dose oral toxicity study was carried out according to OECD guideline 407 [8]. The animals were divided into four groups of 12 animals each (6 males and 6 females). Group 1 received 5 ml/kg body weight of distilled water and served as control, groups 2,3 and 4 received DDE, ASE (400 mg/kg) and Combination of both the drugs (100mg/kg each, means DDE100 mg/kg+ ASE100 mg/kg) because these doses produced better effect in previous study. The test drugs were administered orally daily for 28 days the same time and observed at least twice daily for morbidity and mortality. Body weights of the animals were evaluated weekly. On the 29th day, after an overnight fasting, the rats were anaesthetized with ether and blood sample for haematological and biochemical analysis were collected into tubes with and without EDTA, respectively. Haemoglobin, haematocrit, red blood cell count, white blood cell count, mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and platelet count were determined using an automatic counter (Sysmex K21, Tokyo, Japan). Biochemical analysis was performed on serum obtained after centrifugation of total blood. Standardized diagnostic kits were used for spectrophotometric determination of the following biochemical parameters: SGOT, SGPT, Cholesterol, TG, HDL, LDL, creatinine, alkaline phosphatase and urea.

III. RESULTS AND DISCUSSION

In the present study, related hematological study, only combination (100mg/kg) significantly ($p>0.05$) decreased the RBC count and HCT value, significantly ($p>0.05$) increased the MCH and Platelets count, *Delphinium denudatum* extract (DDE) (400mg/kg) significantly ($p>0.05$) decreased the HCT only and whereas *Amaranthus spinose* extract (ASE) (400mg/kg) significantly ($p>0.05$) increased the Platelets count. So Only ASE showed less toxicity compare to Combination of the drugs and DDE as the results are seen in table 1. After 28 days of treatment, there were less treatment-related changes in hematological parameters between control and treated groups, indicating that the combination of the drug produces erythrocytosis (increases the nor of RBC) like condition because it increases the HCT level indicating increased nor of RBC also size of RBS which is reflected by increased level of MCV. Combination of the drug also increased the number of platelets count. The test drugs DDE, ASE Combination of both the drugs did not produce any serious type of toxicity. The hematopoietic system is one of the most sensitive targets of toxic compounds and is an important index of physiological and pathological status in Human and animals [9].

Table 1: Effect of DDE, ASE and combination of the drugs on hematological analysis.

S.N.	GROUPS	WBC (10^3/µl)	RBC (10^3/µl)	Hb (g/dl)	HCT (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	PLT (10^3/µl)
1	Control (5ml/kg)	7.36±1.12	6.83±0.92	15.01±2.23	54.9±1.116	62.2±2.09	17.00±1.29	27.3±2.20	445±3.01
2	DDE (400 mg/kg, p.o.)	7.4±0.92	6.92±0.31	13.50±1.01	51.40±2.9148	63.2±1.63	17.20±0.29	27.7±2.31	433±0.12
3	ASE (400 mg/kg, p.o.)	7.72±1.96	5.97±0.39	14.44±2.02	50.4±1.92	61.6±2.91	18.1±0.93	28.6±2.01	606±1.21*
4	Combination(100 mg/kg, p.o.)	8.09±0.04	7.87±1.23*	14.10±2.20	58.8±2.9*	65.3±1.19*	17.9±1.27	27.4±4.91	652±1.43*

All values are mean ± SEM . Statistical analysis of data was carried out by one -way ANOVA followed by Dunnett's test, ns = not significant, .*P<0.05, **P<0.01and ***P<0.001 when compared with control group.

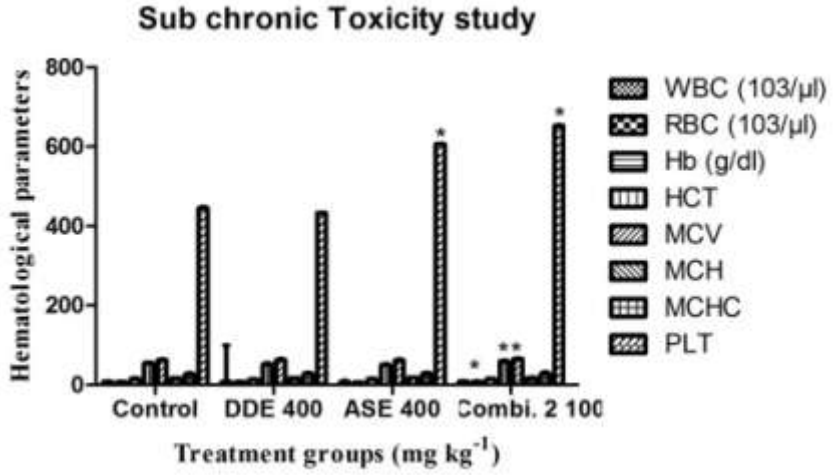

Figure 1. Showing the effect of DDE, ASE and combination of both the drugs on hematological analysis.

In the present study, related to SGOT and SGPT, only combination (100mg/kg) significantly (p>0.05) increased the level of SGOT and SGPT, DDE (400mg/kg) ASE (400mg/kg) and combination (100mg/kg) more significantly (p>0.001) decreased the level of cholesterol. ASE significantly (p>0.05) increased the level of HDL whereas DDE (400mg/kg) and combination of both the drugs (100mg/kg) more significantly (p>0.01) increased the level of HDL which is good indication for health. The test drugs DDE, ASE and combination of the drugs did not produced any significant effect related to the Urea (URA), creatinine (CRTN) and alkaline phosphate (ALP) level as the results are seen in table 2. In addition, most of the biochemical parameters were not also altered by the test drugs, only the combination of both the drug significantly altered in the levels of SGOT and SGPT and increased relative weight of the liver which are not good indicators of liver functions, suggests that sub-chronic administration, slightly altered hepatocytes of rats may disturbed the normal metabolism of the animals. The DDE, ASE and combination of both the drugs more significantly decreased cholesterol levels but increased the HDL level which a good sign of cardiovascular activity, the test drugs may be helpful in treating the stress, amnesia and CVS disorders etc.

Table 2. Effect of DDE, ASE and combination of the drugs on biochemical analysis.

S. N.	Groups	SGOT	SGPT	CHOL	TG	HDL	LDL	URA	CRTN	ALP
1	CONTROL (5ML/KG)	96.00±5.74	30.86±2.14	53.43±3.04	36.14±3.08	1.04±0.03	18.50±0.67	44.43±4.00	1.409±0.14	127.3±5.70
2	DDE (400 MG/KG, P.O.)	101.4±3.39	30.86±1.87	27.00±2.13***	30.74±1.89	1.57±0.07**	17.87±0.94	38.71±1.78	1.38±0.15	137.0±5.66
3	ASE (400 MG/KG, P.O.)	80.86±3.11	31.14±1.55	22.14±1.51***	32.71±1.55	1.45±0.13*	16.67±1.11	42.00±2.54	1.641±0.21	124.6±4.69
4	COMBINATION (100 MG/KG, P.O.)	108.6±3.47*	40.51±3.79*	22.14±3.54***	44.71±3.27	1.57±0.11**	20.83±1.60	39.57±2.82	1.07±0.06	126.7±4.83

All values are mean ± SEM. Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's test, ns = not significant, *P<0.05, **P<0.01and ***P<0.001 when compared with control group

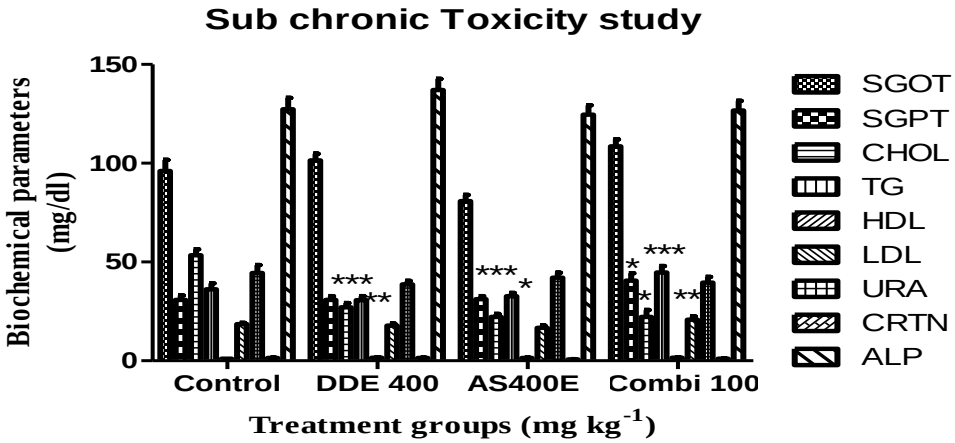

Figure 2. Showing the effect of DDE, ASE and combination of both the drugs on biochemical analysis.

In this study, no significant differences in body weight were observed between control and treated groups during this period as the results are seen in table 3.

Table 3: Mean body weight of rats after 28 days treatment with DDE, ASE and combination of both the drugs.

	MEAN BODY WEIGHT OF THE ANIMALS			
WEEKS	**CONTROL**	**DDE 400mg/kg**	**ASE 400mg/kg**	**COMBINATION OF BOTH THE DRUGS 100mg/kg**
0	128±30.2	130±5.26	127±3.71	135±6.01
1	131±4.12	135±3.97	131±2.41	139±4.11
2	136±5.09	140±2.99	134±4.09	142±3.24
3	141±3.29	143±3.14	141±3.77	146±5.60
4	143±3.91	145±9.21	145±7.21	150±6.3

All values are mean ± SEM . Statistical analysis of data was carried out by one -way ANOVA followed by Dunnett's test, ns = not significant, *P<0.05, **P<0.01and ***P<0.001 when compared with control group.

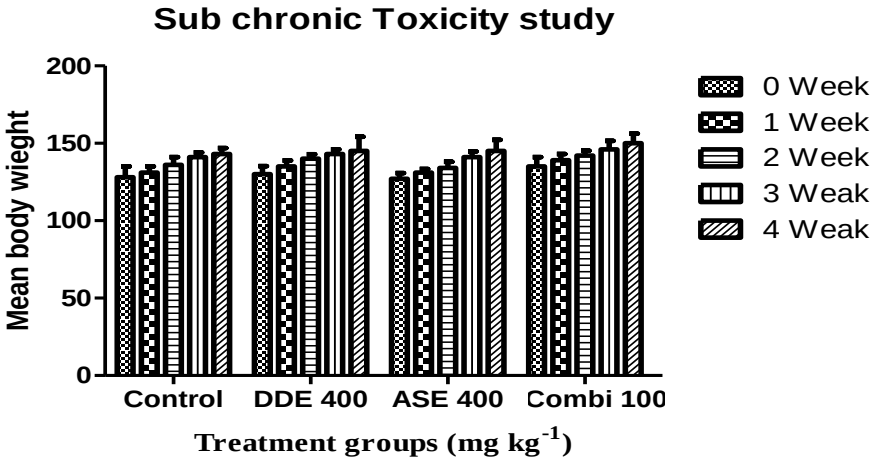

Figure 2. Showing the effect of DDE, ASE and combination of both the drugs on mean body weight.

The recent study showed that the weight of liver in the group treated with combination (100mg/kg) significantly higher ($p < 0.05$) than that of the control group and may produced some types of abnormality. For other organs and other groups there were no changes in organ weight as the results are seen in table 4.

Table 4: Mean organ weight of rats after 28 days treatment with DDE, ASE and combination of both the drugs.

Organs	**Control groups**	**DDE 400mg/kg**	**ASE 400mg/kg**	**Combination of both the drugs 100mg/kg**
HEART	0.31±0.03	0.29±1.01	0.27±0.41	0.32±0.02
LIVER	2.79±0.39	2.83±1.03	3.13±0.51	3.21±0.81*
SPLEEN	0.51±0.13	0.19±0.05	0.14±0.33	0.14±1.11
KIDNEY	0.51±0.13	0.51±1.05	0.47±0.210	.52±1.19
TESTIS	1.27±0.07	1.11±0.31	1.19±0.41	1.21±0.03

All values are mean ± SEM . Statistical analysis of data was carried out by one -way ANOVA followed by Dunnett's test, ns = not significant, *P<0.05, **P<0.01and ***P<0.001 when compared with control group.

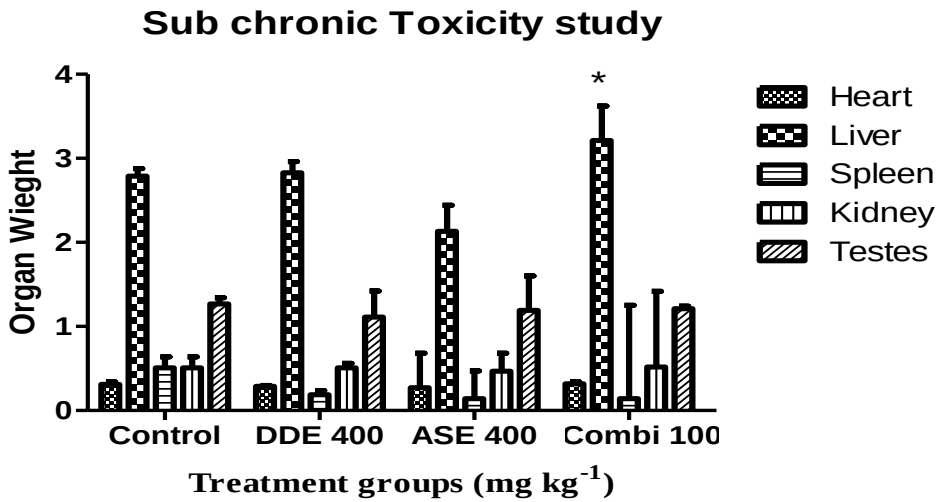

Figure 2. Showing the effect of DDE, ASE and combination of both the drugs on mean body organ weight.

However, it could be argued that these changes may not be toxicologically significant, as they were not corroborated by the biochemical findings (ALT, AST, alkaline phosphatase and glucose). Further more specific assays of toxicity could furnish more information regarding the hepatotoxicity of the combination of the drug.

IV. CONCLUSION

In the recent study it was found that both the drugs extracts singly showed lesser amount of toxicity but in combination of both extract produced little more amount of toxicity compare to control group. However, it could be argued that these changes may not be toxicologically significant and no mortality of animals was observed throughout the study period.

Further more specific assays of toxicity would be done to find out the types and severity of toxicity.

ACKNOWLEDGEMENT

The authors are grateful to Dr. R.M. Dubey, Vice Chancelor of IFTMU and Dr. AK. Ghosh Director of SPS (Schoolof pharmacuitical sciences) for providing constant encouragement, valuable insight and facilities at all stages of this work.

REFERENCES

[1]. K Polasa, and K Nirmala. Ginger: Its Role in Xenobiotic Metabolism. ICMR Bull, 2003; 33: Suppl 6: 57-63.

[2]. J.F Deng. Clinical Toxicity of Herbal Medicine in Taiwan. Proceedings of the 7th International Conference on Health Problems Related to the Chinese in North America, July 1-3, 1994, New York, USA.

[3]. N Qudsia, & M.A. Jafri. Unani drug, Jadwar (*Delphinium denudatum* Wall.) - A review. *Indian* Journal of Traditional Knowledge, 2006; 5(4): 463-467.

[4]. S Zafar, M.A. Aftab, & TA. Siddiqui. Jadwar (*Delphinium denudatum* Wall.) Roots – A Boon in Unani Medicine. *Hamdard Med*, 6(2), 2003, 9-14.
[5]. SZ Rahman, RA Khan& Kumar. A Pharmacological study of *Delphinium denudatum* Wall in morphine deaddiction. J Neurochem; 102 (1), 2007, 142- 143.
[6]. Mishra, SB, Verma A., Mukerjee A and Vijayakumar M. *Amaranthus spinosus L*. (Amaranthaceae) leaf extract attenuates streptozotocin-nicotinamide induced diabetes and oxidative stress in albino rats. A histopathological analysis. Asian Pacific J of Trop Biomed, (2012); 1647-1652.
[7]. P.K Mitra. Comparative Evaluation of Anti Gastric Ulcer Activity of Root, Stem and Leaves of *Amaranthus spinosus Linn*. in Rats. *Int J of Herbal med*, 1(2), 2013, 1675-1680.
[8]. OECD. Repeated dose oral toxicity test method. In:OECD Guidelines for testing of chemicals, N°407. Organization for Economic Cooperation and Development, Paris, France; 2008.
[9]. AA Adeneye,OPAjagbonna, TI Adeleke, Bello SO. Preliminary toxicity and phytochemical studies of the stem bark aqueous extract of Musanga cecropioides in rats. J Ethnopharmacol, 2006, 105: 374-379.

IOSR Journal of Pharmacy (IOSR-PHR) is UGC approved Journal with Sl. No. 5012

M Abid Subchronic Toxicity Study of Hydroethanolic Extract of Delphinium Denudetum Root And Amaranthus Spinosus Leaves on Experimental Rats." IOSR Journal of Pharmacy (IOSRPHR), vol. 7, no. 10, 2017, pp. 20-25.