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Evaluation of the hepatotoxicity of the anti-diabetic drug Diarid: An ayurvedic formulation in white swiss albino mice

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ABSTRACT

Diarid is an Ayurvedic antidiabetic drug. Questions and concerns are being raised nowadays on such Ayurveda formulations with their composition for safety aspects. Though, these are being used safely in without any noticeable untoward effects; there is a need to generate scientific evidence that these are safe and non-toxic. The drugs can usually be detoxified, but some of them can be bio activated become more toxic. The liver often the target organ, most toxicants enter the body through the gastrointestinal tract and after absorption they are carried by the hepatic portal vein in the liver. The toxicology of the liver is complicated by the variety of liver injuries and by the different mechanisms through which the injuries are induced. In the present study, the safety profile of Diarid is tested for acute toxicity, Diarid was administered at a maximal dose of 600 mg/kg to overnight fasted rats and observed closely for behavioural changes, signs of toxicity and mortality if any, continuously for the first six hours and thereafter periodically up to 45 days. Animals were sacrificed on the 46th day. Biochemical parameters and were studied in both serum and liver tissue. In acute toxicity, Diarid at the dose of 600 mg/kg did not produce any observable toxic effects or mortality. No pathological changes on different biochemical parameters in serum and liver homogenate. Based on these observations, it can be concluded that Diarid is safe at therapeutic dose levels.

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INTRODUCTION

Diabetes mellitus Presents with a group of metabolic disorders and there relative or absolute lack of production of Insulin by the body which results in hyperglycemia. This is a chronic condition. Among the two major types of diabetes mellitus Type 1 and Type 2, type 1 generally seen in children and prevalence is 5% to 10% in diabetic population, previously known as juvenile diabetes, is the result of the body's failure to produce enough insulin (Centers for Disease Control and Prevention. National Diabetes Fact Sheet. United States., 2005). Type 2 diabetes is a result of insulin resistance; the body cannot have used the insulin appropriately produced. Prevalence is more, and generally, this condition is diagnosed in adults According to the World Health Organization, recent figures suggest that there are more than 180 million people with diabetes worldwide and this number will double by 2030 (World Health Organization. Diabetes fact sheet, No. 312., 2006). Many millions of people are unaware that they have this disease and remains untreated. Variety of clinical complications such as cardiopathy, nephropathy, and retinopathy, neuropathy is associated with diabetes due to chronic hyperglycemia. If the condition is not managed properly which can lead to life-

threatening complications such as heart disease, stroke, blindness, and kidney failure. Diabetes is the sixth leading cause of death in the U.S. (Danaei et al., 2011). Much attention was not received for hepatotoxicity unlike other complications prevalent in diabetes until the clinical complications emerged due to the anti-diabetic drug therapy in modern medicine. Patients who take anti-diabetic drugs have a high incidence of hepatotoxicity. Underlying mechanisms or predisposing factors for hepatotoxicity in diabetes still unclear. It is recommended that liver enzymes such as alanine aminotransferase should be monitored in patients with diabetes receiving anti-diabetic drugs for which incidences of hepatotoxicity are already reported (Vagula M & Devi SS., 2008).

The liver is one of the most important organs in the body with diverse functions such as metabolism, secretion, storage, and detoxification of endogenous and exogenous substances. The metabolic activities of the liver are by means of various biochemical pathways including hydrolysis, Any disorders in these pathways will lead to the injury of the liver tissue that we call as hepatotoxicity which in turn leads to many life-threatening diseases which results in high mortality worldwide (Madrigal-Santillán E et al., 2014). Hepatotoxicity can be due to medicines, chemicals, dietary disturbances or herb-induced liver damage via hepatotoxins. Hepatic diseases exist as main threats, and this remains unsolved problems worldwide in public health. In spite of enormous advances in modern medicine, hepatic-toxicity exists with the patient, who takes anti-diabetic drugs. Thus, it is needed to identify pharmaceutical alternatives for the treatment of diabetes. The alternatives should be more effective and less toxic. The use of some plants in herbal medicines have played basic roles in human health care, and several scientific researchers have identified these beneficial effects are attributed to the presence of some chemical compounds called phytochemicals. According to WHO, 80% of the world population in the developing countries adopts herbal medicines for their basic health care. Modern medicine can offer only a very limited range of drugs and therapies. So all over the world use of herbal remedies is gaining popularity. Herbal medicines are easy to procure and easy to make formulation. These therapies are fortunately very efficacious and cost-effective. The research world for the constant search of better drug, more potent active principles of the plant, and more palatable formulations for diabetes (Srivastava R& Srivastava P., 2018)

In this view, every effort has been taken to collect and compile the details regarding a drug Diarid, which will be useful to society to venture into an alternative system of medicine. In order to assess the safety or a drug, various studies were carried out in animal-like, mice, rats, guinea pigs, dogs and monkeys under the varying condition of drug administration. After the establishment of pharmacological activity in experimental animals, toxicity studies are carried out in human models. Thus it is normally done to evaluate the safety of a new found plant material (extract, active factor or a compound) or a newer combination of herbal medicine. Ayurveda utilises natural resources of plant, animal, metal and mineral origin in therapeutics of different pathologies. These resources are converted into formulations based upon the need by following specified classical guidelines. Herbomineral and metallic formulations are an important part of Ayurveda that is attributed to be safe and efficacious when manufactured and used judiciously. *Diarid*, is an anti-diabetic drug which contains0.375 gms of Ekanayakam (Salacia reticulata), maramanjal, (Coscinium fenestratum), manjal (Curcuma longa), venga (Pterocarpus marsupium), paachotti (Symplocos Racemosa), thechi (Ixora coccinea), raamacham (Vetiveria zizanoides), Nellika (Emblica officinalis) Importance of following traditional pharmaceutical procedures in preparation of Ayurvedic formulations have been well-established. So, pharmaceutical researchers screening the herbal products and other natural products for the treatment of diabetes mellitus which is less in toxicity and also cost-effective. Considering this, safety profiles Diarid was evaluated in the current study. The current study was conducted to evaluate the hepatotoxic effect of Diarid biochemically by administering it to experimental animals.

MATERIALS & METHODS

Animals: 24 adult young, healthy, nulliparous, virgin female Swiss albino mice weighted 150-190 g were selected and acclimatised for 120 days, maintained under the standard laboratory conditions with the proper food and water for the experiment. The animals were divided into 4 groups of six mice in each group. For acute toxicity study was carried out by following Organization for Economic Cooperation and Development (OECD) 425 guidelines in 2001.

Group 1 (n=6): Normal-Normal diet &distilled water was given.

Group 2 (n=6): drug Control-Normal diet & diarid single dose (60mg/kg body weight per day) was given.

Group 3 (n=6): Sub-acute Group-Normal diet &diarid double dose (120mg/kg body weight per day) was given.

Group 4 (n=6): Acute Group-Normal diet & an acute dose of diarid (600mg/kg body weight per

day) was given. After 7-15 days if there was no LD_{50} a new dose of 1.2gm/kg body weight is administered to the 50% of the group.

MATERIALS

Dose and schedule: The therapeutic dose is 60 mg/kg body weight. Rat dose was calculated by referring to a table of Paget and Barnes. As classics advocate using diarid capsule content were mixed with the distilled water was administered orally with the help of oral cannula Contents of the diarid capsule along with water were orally administered at the limit dose of 600 mg/kg to overnight fasted rats. The rats were observed closely for behavioral changes, signs of toxicity, and mortality, if any continuously for the first six hours' drug administered for 45 days. Animals were fasted overnight, weighed.

Blood collection: Supraorbital plexus was punctured, and blood was collected using capillaries in two different tubes, one containing anticoagulant fluid for hematological parameters and another plain tube for serum biochemical investigations. Then the rats were sacrificed with an overdose of diethyl ether, and the abdomen was opened through midline incision to observe the autopsy changes followed by dissecting out the important organs.

Preparation of Liver homogenate: Liver tissues were removed to ice –cold containers for extraction and for estimations. Accurately weighed 100mg liver tissue was ground in a mortar with a pestle under cold condition. A 10% homogenate was prepared. By adding 9 volumes, i.e. 0.9ml of sodium carbonate for ALP (sodium bicarbonate buffer, pH 10), for AST&ALT, 0.9 ml of phosphate buffer pH 7.4, FOR glutathione reductase 0.9 ml of phosphate buffer (o.1 m) pH 7.4, TBARS 0, 9ml, 1 ml of homogenate used of TRIS-HCL BUFFER. Centrifuges at 2000rpm.The supernatant was used for the assay of enzymes

Biochemical parameters: Biochemical parameters in serum and liver were analysed by using the semi-automated biochemical analyser. The studied parameters were serum glutamate pyruvate transaminase, (SGPT), Serum glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP), Glutathione reductase, TBARS

Study setting: The present study was conducted at the Department of Biochemistry, School of Medical Education, Kottayam, Kerala, India

Ethical consideration: The study was approved by the Institutional animal ethical committee, school of medical education, Kottayam, India

Data analysis: Data were analyzed using SPSS.15 versions. To observe the differences between the two groups students' t-test was used. A p value less than 0.05 considered as significant.

RESULTS

Table 1: Aspartate aminotransferase activity in serum (IU/L) and liver (IU/100gm wet tissue)

Groups	Serum	liver
I. Normal control	23.84±2.84	39.7±2.43
II. single dose	20.95±1.826	41.8±2.404
III. double dose	26.7±2.17	42.4±2.505
IV. acute dose	27.05±2.23	42.75±2.425

Group II, Group III and Group IV compared with group I. Values expressed as mean ± SD of 6 rats

Table 1 presents the mean values of Aspartate aminotransferase activity in serum (IU/L) and liver (IU/100gm wet tissue) in the control and experimental group.

Table 2: Aspartate aminotransferase activity in serum (IU/L) and liver (IU/100gm wet tissue)

Comparison between groups	t values		
	Serum	Liver	P values
I&II	0.181	0.347	All Values
I&III	1.77	0.882	> 0.05
I&IV	1.90	2.15	

Group II, Group III and Group IV compared with group I. Values expressed as mean ± SD of 6 rats, p-value >0.05 not statistically significant value ≤ 0.05 is statistically significant

Table 2 presents a comparison of Aspartate aminotransferase activity in serum (IU/L) and liver (IU/100gm wet tissue) in the control and experimental group. P values >0.05 so no statistical difference.

Table 3 presents the mean values of Alanine transaminase activity in serum (IU/L) and Liver (IU/100gm wet tissue) in the control and experimental group.

Table 3: Alanine transaminase activity in serum (IU/L) and Liver (IU/100gm wet tissue)

Groups	Serum	Liver
I. Normal control	20.01±2.39	62.27±1.32
II. Single Dose	18.35± 2.01	61.96±2.41
III. Double Dose	23.05±2.425	63.1±3.80
IV. Acute dose	23.15±2.525	63.9±4.71

Group II, Group III and Group IV compared with group I. Values expressed as mean ± SD of 6 rats, p-value >0.05 not statistically significant value ≤ 0.05 is statistically significant

Table 4: Alanine transaminase activity in serum (IU/L) and Liver (IU/100gm wet tissue Comparison between controls and study group

Comparison between groups	t values		
	Serum	Liver	P values
I&II	1.427	0.276	All Values
I&III	2.188	0.505	> 0.05
I&IV	2.213	0.8170	

Group II, Group III and Group IV compared with group I. Values expressed as mean ± SD of 6 rats, p value >0.05 not statistically significant value ≤ 0.05 is statistically significant

Table 4 presents a comparison of Alanine transaminase activity in serum (IU/L) and Liver (IU/100gm wet tissue) in control and experimental group p values >0.05 so no statistical difference.

Table 5: Alkaline phosphatase activity in liver (IU/mg protein) and serum (IU/L)

Groups	Liver	Serum
I.Normal control	1.55±0.1142	25.24±1.3894
II. Single Dose	1.45±0.495	24.43±1.1946
III. Double Dose	1.68±0.160	23.5±2.205
IV. Acute dose	1.75±0.385	26.47±1.3346

Group II, Group III and Group IV compared with group I. Values expressed as mean ± SD of 6 rats

Table 5 presents the mean values of Alkaline phosphatase activity in liver (IU/mg protein) and serum (IU/L) in the control and experimental group.

Table 6: Alkaline phosphatase activity in liver (IU/mg protein) and serum (IU/L).Comparison between controls and study group

Comparison between groups	t values		
	Serum	Liver	P values
I&II	1.088	0.4830	All Values
I&III	1.636	1.625	> 0.05
I&IV	1.5652	1.2210	

Group II, Group III and Group IV compared with group I. Values expressed as mean ± SD of 6 rats, p-value >0.05 not statistically significant value ≤ 0.05 is statistically significant

Table 6 presents a comparison of Alkaline phosphatase activity in liver (IU/mg protein) and serum (IU/L) in control and experimental group p values >0.05 so no statistical difference.

Table 7 presents the mean values of Activity of Glutathione reductase in liver (10 ^{2}µ mols NADPH oxidized /mt and blood (IU/gm) in the control and experimental group.

Table 7: Activity of Glutathione reductase in liver (10 2µ mols NADPH oxidized /mt0 and blood (IU/gm)

Groups	Serum	Liver
I. Normal control	30.05 ± 1.47	6.75 ± 0.164
II. Single Dose	30.62 ± 2.78	6.95 ± 0.31
III. Double Dose	29.18 ± 2.95	7.15 ± 0.454
IV. Acute dose	30.16 ± 2.25	7.25 ± 0.675

Group II, Group III and Group IV compared with group I. Values expressed as mean ± SD of 6 rats

Table 8: Activity of Glutathione reductase in liver (10 2µ mols NADPH oxidized /mt and blood (IU/gm). Comparison between controls and study group

Comparison between groups	t values		P values
	Serum	Liver	For All
I & II	0.4453	1.398	comparisons
I & III	0.647	2.040	p value>0.05
I & IV	0.1009	1.398	

Group II, Group III and Group IV compared with group I. Values expressed as mean ± SD of 6 rats, p value >0.05 not statistically significant value ≤ 0.05 is statistically significant

Table 8 presents a comparison of Activity of Glutathione reductase in liver (10 ^{2}µ mols NADPH oxidized /mt and blood (IU/gm) in the control and experimental group. p values >0.05 so no statistical difference

Table 9: Activity of TBARS in liver (mmols/mg protein) and serum (µ mols/ ml)

Groups	Serum	Liver
I. Normal control	5.05±0.68	11.25±0.825
II. Single Dose	4.15±0.875	11.93±0.825
III. double dose	4.75±0.725	10.65±0.938
IV. acute dose	5.373±0.585	10.59±1.185

Group II, Group III and Group IV compared with group I. Values expressed as mean ± SD of 6

Table 9 presents the mean values of Activity of TBARS in liver (mmol/mg protein) and serum (µ mols/ ml)- in control and experimental group.

Table 10: Activity of TBARS in liver (mmols/mg protein) and serum (µ mols/ ml).Comparison between controls and study group

Comparison between groups	t values		
	Serum	Liver	P values
I & II	1.480	1.695	All values
I & III	1.477	1.773	> 0.05
I & IV	0.755	1.120	

Group II, Group III and Group IV compared with group I. Values expressed as mean ± SD of 6 rats, p value >0.05 not statistically significant value ≤ 0.05 is statistically significant

Table 10 presents a comparison of Activity of TBARS in liver (mmol/mg protein) and serum (µ mols/ ml)- in control and experimental.

In this study, there is no significant elevation or change in levels of liver enzymes in both the con-

trol and experimental group. So Diarid, the antidiabetic drug does not have any hepatotoxic effect on the liver.

DISCUSSION

According to the International Diabetic Federation (IDF) in India, 4.1 crores are suffering from DM; it is expected to increase by 7 crores in 2025 (Eggadi et al., 2014). Herbal medicines are known for antidiabetic activity since ancient years. The plant also contains substances which are toxic to humans. So it is very important to study the toxicity of herbal products which contains various parts of plants. So in this present study Diarid, antidiabetic product in the market is choosing to know the side effects by analysing the hepatotoxicity after administering to white albino female rats. Female rats were preferred in studies for acute toxicity because female rats are more sensitive (Lipnick., 1995). The results of acute toxicity study provide some important information for determining the essential dosage of drugs in animal studies and also to determine the tolerable dose.

In this study, the toxic effects of the ayurvedic drug Diarid on the liver were evaluated biochemically by acute and subacute procedures. In sub-acute method single dose of 60mg/kg body wt./day and double doses of 120mg/kg body wt/day drug was administered. In the acute method, 600mg/kg/body wt was administered. The drug administered for 45 days. There is no significant effect on body weight, which reveals the physiological and metabolic status of animal (Pariyani et al., 2015 pp45)

The results showed that there is no weight loss in Diarid fed rats. On biochemical analysis, there is no significant elevation of enzymes AST, ALT in both serum and liver tissue homogenate indicating there is no hepatocellular or obstructive damage due to the drug. The major Thiobarbituric acid reactive substance (TBARS)in the body is malonaldehyde (MDA). Malonaldehyde is an end product of lipid peroxidation. In liver homogenate and serum there was no significant elevation of TBARS suggesting no peroxidative changes. Glutathione reductase is an antioxidant enzyme shows no significant variation in both serum and liver homogenate. The present study reveals that at the Diarid does not show any acute toxicity symptoms in rats. This report is the first one regarding the drug Diarid which is the antidiabetic drug produced by Nagarjuna pharmacy. No other literature exists regarding the hepatotoxicity of Diarid.

Ayurvedic drugs is a safer alternative to the side effects of chemical medicines. Some adverse effects of modern drugs result in search of natural herbs and thus bringing the traditional medicine systems into the limelight. Still, there are questions regarding the efficacy and toxicity of medicines, and there is a perception that Ayurveda does not have protocols to test the toxicology. But this may be the earliest system with well-developed have developed the discipline of toxicology. Toxicology or *Agada Tantra* is one among the eight clinical specialities of Ayurveda for thousands of years. We can say that Ayurvedic physicians must develop a clear perspective of the safety of Ayurvedic medicines and treatments and find solutions from within. The Ayurvedic toxicologist well trained in *Agada Tantra* seems to be the answer. All potential hazards associated with Ayurvedic treatments should be listed, guidance on how to anticipate them and also manage them would be the first step in this direction (P. Ram Manohar., 2014)

CONCLUSION

The present study provides evidence that the drug Diarid, an antidiabetic drug which is ayurvedic formulation produced by the Nagarjuna Pharmacy, Thodupuzha, Kerala have no hepatotoxic effect on experimental mice. This is an acute toxicity study, so we also recommend chronic toxicity study. More studies should be conducted, and liver enzymes should monitor in human population who use this drug to understand it's effectivity.

REFERENCES

Centres for Disease Control and Prevention (2005). National Diabetes Fact Sheet. The United States, 2005. www.cdc.gov/diabetes/pubs/pdf/ndfs_2005.pdf. Accessed February 12, 2008.

Danaei G, Finucane MM, Lu Y, Singh GM, Cowan MJ, Paciorek CJ, et al. (2011). National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2.7 million participants. Lancet .378: 31–40.

Eggadi VP, Kankanala S, Bandaru S, Kulindaivelu, Jupally V (2014). Investigation of lipid profile and ocular oxidative stress of Chloroxylon swietenia on Streptozotocin –nicotinamide-induced diabetic rats. Int J Green Pharm.8 (2): 90-96.

Lipnick RL, Cotruvo JA, Hill RN, Bruce RD, Stitzel KA, Walker AP, et al. (1995). Comparison of the up and down conventional LD 50, and fixed acute toxicity procedures. Fd. Chem. Toxico. 33:223-231.

Madrigal-Santillán E et al. (2014). World J Gastroenterol. 20 (40): 14787-14804

Organisation for Economic Co-operation and Development (OECD) Guideline No. 423 (2001). Acute oral toxicity in animals. OECD/OCDE No. 425. Available from: http://www.ntp.niehs.nih.gov/iccvam/ suppdocs/feddocs/oecd/oecd_gl 423.pdf.

Paget GE, Barnes JM (1964). Evaluation of drug activities. In: Laurence DR, Bacharach AL, editors. Pharmacometrics. London: Academic Press. 1:50.

Pariyani R, Intan SI, Amalina AA, Faridah A, Khozirah S, Mohd RS (2015). Phytochemical screening and acute oral toxicity study of java tea leaf extracts. Bio Med Research International:1-8.

Ram Manohar P (2014). Toxicity of Ayurveda medicines and safety concerns: The need to revive the branch of toxicology in Ayurveda. Anc Sci Life.34 (1): 1–2.10.4103/0257-7941.150761.

Srivastava R, Srivastava P (2018) Hepatotoxicity and the Role of Some Herbal Hepatoprotective Plants in Present Scenario. GJ Dig Dis.3 (2):2

Vagula M, Devi SS (2008). Hepatotoxicity of Antidiabetic Drugs.US Pharm. 33 (5):3-9.2

World Health Organization. Diabetes fact sheet No.312 (2006). www.who.int/mediacentre/factsheets/fs312/en/index.html.