

Withania Somnifera: Correlation of Phytoconstituents with Hypolipidemic and Cardioprotective Activities

Subasini Uthirapathy¹ and Tara Fuad Tahir²

¹Department of Pharmacology, Faculty of Pharmacy, Tishk International University, Erbil, Kurdistan Region - F.R. Iraq

²Department of Medical Microbiology, Faculty of Science and Health, Koya University, Koya KOY45, Kurdistan Region - F.R. Iraq

Abstract—*Withania somnifera* (WS) (Dunal) or Ashwagandha is a well-known hypolipidemic herb and antioxidant. In this study, 75% ethanolic extract of WS is attempted to evaluate the cardioprotective activity of isoproterenol-induced cardiotoxicity and hypolipidemic activity in Triton WR 1339-induced hyperlipidemia. In addition, phytochemical evaluation of the same extracts analyzed by gas chromatography–mass spectrometer (GC–MS). This study found that 7 days of therapy with WS extracts at 1000 mg/kg b.wt. reduced cholesterol by 76%, low-density lipoprotein (LDL) by 71%, and TAG by 12% ($P < 0.05$). Furthermore, it can significantly reduce cholesterol and LDL levels ($P < 0.05$). Similarly, the use of 50 mg/kg b.wt. of WS extract showed a cardioprotective effect against isoproterenol-induced cardiac toxic rats. The antioxidants glutathione, glutathione peroxidase, and catalase are increased in WS extract ($P < 0.05$), whereas the release of cardiac indicators in heart tissue is reduced ($P < 0.05$). Furthermore, a 30-day treatment with WS also reduced triacylglycerol in isoprenaline-induced cardiotoxic rats. GC–MS analysis of the methanol fraction of the Ashwagandha 70% ethanolic extract showed the presence of higher concentrations of fatty acids. In conclusion, WS showed hypolipidemic and cardioprotective activities in diseased animals induced by isoproterenol and Triton WR 1339.

Index Terms—Antioxidants, Cardiac markers, Gas chromatography–mass spectrometer, Isoproterenol, Lipid, Triton WR 1339.

I. INTRODUCTION

Withania somnifera (Dunal) (WS) is commonly known as winter cherry. It is a green shrub belonging to the Solanaceae family. Earlier references have shown that the plant preparations have anti-inflammatory (Boehm et al., 2000), anti-cancer, anti-stress, and immunomodulatory activities. WS has also shown reports of the central nervous system

(CNS), endocrine (Subasini et al., 2007), and cardiovascular (Jasemi et al., 2020) diseases. Furthermore, WS preparations are thought to affect GABAergic (gamma-aminobutyric acid (GABA) (Dar et al., 2016) or cholinergic (Kumari et al., 2020) neurotransmission, which could be associated to a variety of CNS disorders. Other plant parts have been utilized to treat various diseases for a long time.

The root of WS is the central part of the plant for its treatment. WS root powder at 0.75 g and 1.5 g/rodent/day can significantly reduce the total cholesterol and triglycerides (TGLs) in plasma (Nishant and Narasimhacharya, 2006). Then, a considerable increase in plasma high-density lipoprotein (HDL) cholesterol levels, the action of 3-Hydroxy-3-Methyl-Glutaryl-CoA reductase (HMG-CoA) and liver bile acids was observed in these rats. A similar pattern was also observed in the excretion of bile acids, cholesterol, and neutral sterols in hypercholesterolemic rats with oral administration of WS. Further, a significant decrease in lipid-peroxidation occurred in WS administered hypercholesterolemia animals when compared to their normal group of rats. However, it gives an idea that WS root extract is also attractive for lowering the lipid profile (Saggam et al., 2021).

It is familiar that WS has the ability to control oxidative stress markers in the body. Conclusively, the root extract decreases lipid peroxidation and boosts superoxide dismutase (SOD) and catalase activity (Adams et al., 2002). As a result, it has free radical scavenging activity. Furthermore, the hypolipidemic (Nishant and Narasimhacharya, 2006) and antioxidant activity of WS has been demonstrated by Priscilla and Prince, 2009. We attempted to assess the cardioprotective impact, as well as hypolipidemic and antioxidant activities, in isoproterenol-induced cardiotoxicity and Triton WR 1339-induced hyperlipidemia in this research. To correlate the pharmacological activity with its active constituents, gas chromatography–mass spectrometer (GC–MS) analysis also carried out.

II. MATERIALS AND METHODS

A. Chemicals and Reagents

Isoproterenol, malondialdehyde, 1,1'-diphenyl-2-picrylhydrazyl, and 2,2'-azinobis-(3-ethyl-benzothiazoline-

ARO-The Scientific Journal of Koya University
Vol. IX, No. 2 (2021), Article ID: ARO.10844, 7 pages
DOI:10.14500/aro.10844

Received: 28 July 2021; Acceptance: 25 September 2021

Regular research paper: Published: 15 October 2021

Corresponding author's e-mail: tara.fuad@koyauniversity.org

Copyright © 2021 Subasini Uthirapathy, Tara Fuad Tahir. This is

an open-access article distributed under the Creative Commons Attribution License.



6-sulfonic acid) were purchased from Sigma Chemical Co., St. Louis, MO, USA. Biochemical kits for the assay of cholesterol, TGLs, and HDL cholesterol were procured from Randox Laboratories. Creatine kinase (CK)-MB assay kit was purchased from SPINREACT. All the other reagents used were of analytical grade.

B. Collection and Identification of Plants

The root of WS was obtained from Madurai. The plant material was identified in the Department of Pharmacognosy, Centre for Advanced Research of the Indian System Medicine, SASTRA University, Thanjavur, Tamil Nadu, India. The voucher is saved in the same department, document number (0064).

C. Preparation of Extract

The collected plant material was dried for 15 days a room temperature. The raw materials were grinded to use it for extraction with 70% ethanol and the cold infiltration method was used.

Vacuum rotary evaporator was used to concentrate the extract 40°C. Refrigeration was used to store the concentrated extract of 3.83% as the extract yield.

D. Experimental animals

Male Wistar albino rats weighing 180–220 g were allowed to have standard pellets from Hindustan Lever Foods, Bengaluru, India, and water *ad libitum*. They are placed under normal environmental conditions. All rats experiments were performed after obtaining animal ethics approval (Authorization No. 7/SASTRA/IAEC/RPP).

E. Hypolipidemic Activity (Acute Model)

Triton WR 1339 is a non-ionic detergent which has been used to induce hyperlipidemia in animals. Animals were divided into five groups of six rats each. Group 1 animals considered as normal and were fed with standard diet. Group 2 animals received Triton WR 1339. Group 3 animals were pre-treated with atorvastatin 10 mg/kg b.wt./day (p.o.) for 7 days. Groups 4 and 5 animals were pre-treated with WS at the doses of 500 and 1000 mg/kg/day (p.o.), respectively, for 7 days. During treatment, on the 5th day, 1 h after the administration of that samples, all animals except Group 5 were injected with Triton WR 1339, intraperitoneally (i.p.) (dissolved in saline) at the single dose of 400 mg/kg b.wt. The animals were fasted for 3 h before administration of Triton WR 1339 and the fasting was continued up to 48 h after administration of Triton WR-1339. All animals were fed with water after the injection of Triton WR 1339 (Majithiya et al., 2004). The blood was collected from all animals before and after 48 h the administration of Triton WR 1339 by retro-orbital puncture under volatile (ether) anesthesia. Plasma total cholesterol, TGL, and low-density lipoprotein (LDL) by Subasini et al., 2019b, were analyzed.

F. Cardioprotective Activity (Chronic Model)

The experimental animals were divided into four groups, with six animals in each group. For the first group, normal

rats were given only a standard diet. Group 2 animals received isoproterenol (ISO). Group 3 animals were pretreated with WS at a dose of 50 mg/kg body weight for 30 days without ISO. Group 4 animals were pretreated with WS 50 mg/kg body weight for 30 days with ISO. On days 31 and 32, animals in Groups 2 and 4 were injected with ISO 85 mg/kg body weight subcutaneously (s.c.) for 2 days. At the end of the study, the animals were sacrificed by cervical dislocation. Blood was collected and then plasma was separated. Heart tissue was removed immediately and washed thoroughly with saline. The cardiac tissue homogenate was prepared in 0.1 M Tris buffer (pH 7.4).

Various biochemical parameters like Creatine Kinase (CK) (Ohkawa et al., 2004), Lactate dehydrogenase (LDH) (King, 1959), Glutamate Pyruvate transaminase (GPT) (Mohun and Cook, 1957), Glutamate Oxaloacetate transaminase (GOT) (Paglia and Valentine, 1967), Thiobarbituric acid Reactive Substances (TBARS) (Ohkawa et al., 2004), Reduced Glutathione (GSH) (Moron et al., 1979), Catalase (Aebi, 1974) were estimated. The plasma concentration of total cholesterol and HDL cholesterol (HDL_c) was determined using an enzyme kit from Randox Laboratories Ltd., UK (Richmond, 1973). First, HDL_c was determined after precipitation of lipoproteins containing apolipoprotein B with dextran sulfate (Warnick et al., 1992). Next, the plasma TGL concentration and homogenate of heart triacylglycerol (TAG) concentration were analyzed by Foster (Foster and Dunn, 1973). Finally, the Friedewald equation (Friedewald et al., 1972) is used to determine the LDL cholesterol (LDL_c) concentration.

G. GC–MS Analysis

Ten milligrams of WS extract were dissolved in methanol. The methanol fraction separated from the 75% ethanol extract was injected for GC–MS analysis. The following experimental conditions to analyze the samples by GC–MS were applied on the PerkinElmer Clarus 500 GC: Elite 5 column (5% biphenyl 95% dimethylpolysiloxane), column size 30 m × 0.32 mm, gas loading – 1 ml/min of helium, column temperature from 50°C to 285°C, a speed of 10°C/min for 5 min, at 285°C, the temperature of the injector, and the detector was 290°C, and the sample is injected split mode, injection volume: 0.5 µl (2 mg/100 ml methanol solution). The total run time was 30 min. Mass spectra were acquired using a PerkinElmer-TurboMass Gold Mass Detector. Transfer line temperature – 230 °C, Source temperature – 230 °C, scan range is from 40 – 450 amu, ionization technique – Electron ionization technique. The component identification was confirmed by comparing mass spectra of compounds with available NIST and Willey mass spectral library. The quantitative composition was obtained by normalizing the peak area (Subasini et al., 2019b).

H. Statistical Analysis

The results are given Mean ±SD, and statistical significance is determined using a one-way analysis of variance using the Duncan's multiple range test. A difference of $P < 0.05$ was

considered significant and the SPSS software (version 12.0) was used for statistical analysis.

III. RESULTS

A. Effect of WS on Triton WR 1339-Induced Hyperlipidemia

Table I shows the results of significant increasing of cholesterol, LDL, and TAG levels ($P < 0.05$) in the rats with Triton WR 1339 administration compared to normal rats. Rats pretreated with extract at 500 mg/kg b.wt. showed no significant hypolipidemic activity, but rats pre-treated with extract at 1000 mg/kg b.wt. for 7 days showed a reduction in cholesterol levels and LDL against a diseased group of rats. Treatment with atorvastatin reduced cholesterol and LDL levels significantly ($P < 0.05$) without changing TAG levels.

B. Effect of WS on Isoproterenol-induced Cardiotoxicity

Isoproterenol administration was observed to raise cholesterol, LDL significantly, and TAG levels in serum while decreasing HDL levels ($P < 0.05$). Treatment of rats with WS extract did not reverse substantially normal cholesterol, LDL, and HDL levels. At a dose of 50 mg/kg b.wt, pre-treating rats with WS extract for 30 days can dramatically reduce TAG levels ($P < 0.05$) in Table II. The level of cardiac markers was considerably higher in the serum of diseased animals and lowered in the heart homogenate ($P < 0.05$). The discharge of cardiac markers was observed to be dramatically reduced ($P < 0.05$) when rats were pre-treated with the extract and the findings are summarized in Table III. In the same way, the amount of TBARS was higher, and the level of antioxidants was found to be lower in the serum and heart homogenate of the diseased group of rats. Pre-treating animals with WS extract can considerably improve antioxidant levels ($P < 0.05$), reduce membrane damage, and reduce the release of TBARS which are shown in Table IV.

C. GC-MS Analysis of WS Extract

GC-MS analysis showed octadecadienoic acid methyl ester and hexadecadienoic acid ethyl ester as the primary

compound. It was followed by glycerine, sucrose, and 4H, 1,2,4-Triazol-3-amine-4-propyl as the primary compound in Fig. 1. Other chemical compounds are mentioned in Table V.

IV. DISCUSSION

The HMG-CoA reductase activity has been reported to have decreased by root powder of WS (Nishant and Narasimhacharya, 2006) at the dose of 750 mg/kg b.wt./day/rat. To evaluate the effect of WS's 70% ethanolic extract, we have selected two different doses with 750 mg/kg b.wt. as the middle dose. Nishant and Narasimhacharya (2006) have administered drug for 4 weeks. In our present study, we have attempted to evaluate the effect of extract for a shorter duration. Thus for our current study, we have administered extract only for 7 days and compared the same with the effect of the standard drug atorvastatin. Peer et al., 2008, have adopted the diet-induced hyperlipidemia method, whose mechanism of action increases HMG-CoA reductase activity. We have also planned to select a hyperlipidemic model related to the activity of that enzyme. Triton WR 1339 is a surfactant that can induce hyperlipidemic conditions by increasing HMG-CoA reductase activity (Badavi et al., 2020). Moreover, Triton WR 1339 is a widely accepted model for evaluating the effect of hypolipidemic herb (Ipseeta et al., 2004).

The activity of WS extract at 1000 mg/kg b.wt. in Triton WR 1339 caused hyperlipidemic rat is comparable to that of the commercially available standard medication atorvastatin. Atorvastatin treatment was observed to significantly decrease cholesterol and LDL ($P < 0.05$) without effect on TAG. Since the Triton WR 1339 increases the activity of HMG-CoA reductase, the decrement observed in atorvastatin treatment might be due to decreasing the activity of HMG-CoA reductase (Youssef et al., 2002). A comparable activity followed by WS treatment shows that the WS extract inhibits HMG-CoA reductase. It was observed a reduction in HMG-CoA reductase activity (Subasini et al., 2014). The hypolipidemic activity of WS might be due to the presence of polyunsaturated fatty acids in chromatogram GC-MS of the chemical compounds of WS extract (Minihane et al., 2005). Isoproterenol-induced cardiotoxic model is adopted

TABLE I
EFFECT OF WS EXTRACT AND ATORVASTATIN IN TRITON WR 1339-INDUCED HYPERLIPIDEMIC RATS

Groups	Treatment	Cholesterol (mg/dl)	LDL (mg/dl)	TAG (mg/dl)
1	Normal rats	63.5±5.3	40.2±10.5	53.8±10.6
2	Triton WR 1339	878.1±18.2*	678.8±22.1*	675.0±43.3*
3	Atorvastatin (10 mg/kg bw)	473.5±7.5 ^a	312.3±3.6 ^a	525.1±12.4 ^{ns}
4	Triton WR 1339+WS (500 mg/kg b.wt.)	754.9±28.3 ^{ns}	580.5±32.5 ^{ns}	562.5±23.9 ^{ns}
5	Triton WR 1339+WS (1000 mg/kg b.wt.)	665.3±19.5 ^a	479.4±19.8 ^a	525.0±14.4 ^{ns}

Values are Mean±SD. n=6. Statistical difference is calculated in one-way ANOVA (LSD) method (least significant difference). *Statistical difference between Group 1 and 2 ($P < 0.05$). ^aSignificant difference between Groups 3, 4, and 5 versus Group 2 ($P < 0.05$). ns – No significant difference between specific groups versus Group 2. LDL and TAG: Triacylglycerol, LDL: Low-density lipoprotein

TABLE II
EFFECT OF WS EXTRACT ON LIPID PROFILE IN ISOPROTERENOL-INDUCED CARDIOTOXIC RATS

Groups	Treatment	Cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	TAG (mg/dl)
1	Normal	74.10±4.1	20.30±0.7	40.40±1.2	66.80±4.6
2	ISO	95.20±2.7*	14.80±0.4*	62.80±1.3*	87.80±2.3*
3	WS (50 mg/kg b.wt.)	77.30±2.4 ^ε	19.70±0.9 ^ε	44.30±2.3 ^ε	66.50±5.1 ^ε
4	ISO+WS (50 mg/kg b.wt.)	86.60±1.7 ^{ns}	16.30±0.7 ^{ns}	58.04±2.3 ^{ns}	61.30±8.9 ^a

Values are Mean±SD. n=6. Statistical difference is calculated in one-way ANOVA method (least significant difference). *Statistical difference between Groups 1 and 2 ($P < 0.05$). ^ε – No significant difference between Group 1 versus Group 3. ns – No significant difference between Group 1 versus Group 4. ^aSignificant difference ($P < 0.05$) between Group 1 versus Group 4. HDL: High-density lipoprotein, LDL: Low-density lipoprotein, TAG: Triacylglycerol

TABLE III
EFFECT OF WS ON CARDIAC MARKERS IN ISOPROTERENOL-INDUCED CARDIOTOXICITY

Sample	Parameter	Normal	ISO	WS	ISO+WS
Plasma	CK	4.500±0.0300	8.300±0.0300*	4.400±0.0200 ^ε	5.800±0.0900
	LDH	0.200±0.0040	0.440±0.0300*	0.240±0.0200 ^ε	0.290±0.0070 ^a
	GOT	0.050±0.0003	0.080±0.0900*	0.050±0.0200 ^ε	0.062±0.0009 ^a
	GPT	0.030±0.0009	0.130±0.5000*	0.040±0.0100 ^ε	0.050±0.0040 ^a
Heart	CK	236.900±4.4000	174.500±3.3000*	239.000±13.0000 ^ε	228.800±5.9000 ^a
	LDH	2.700±0.0400	1.400±0.0600*	2.600±0.0200 ^ε	2.700±0.0900 ^a
	GOT	2.300±0.0300	1.400±0.0170*	2.400±0.0160 ^ε	2.400±0.0900 ^a
	GPT	2.700±0.0600	1.500±0.0300*	2.600±0.0100 ^ε	2.900±0.0600 ^a

Values are Mean±SD. n=6. Statistical difference is calculated in one-way ANOVA method (least significant difference). *Statistical difference between Groups 1 and 2 ($P<0.05$). ^ε – No significant difference between Group 1 versus Group 3; ^asignificant difference ($P<0.05$) between Group 1 versus Group 4. One micrograms of phosphorous liberated/min/mg of protein, 2 µg of pyruvate liberated/min/mg of protein. CK: "Creatine kinase," LDH: Lactate dehydrogenase," GOT: "Glutamate oxaloacetate aminotransferase," GPT: "Glutamate pyruvate transaminase"

TABLE IV
EFFECT OF WS ON ANTIOXIDANTS IN ISOPROTERENOL-INDUCED CARDIOTOXICITY

Sample	Parameter	Normal	ISO	WS	ISO+WS
Plasma	TBARS	0.08±0.0005	0.17±0.0002*	0.08±0.0400 ^ε	0.11±0.0100 ^a
	GSH	4.60±0.0190	2.60±0.0800*	4.20±0.0250 ^ε	4.00±0.0400 ^a
	GPX	3.60±0.0600	2.70±0.1000*	3.80±0.0400 ^ε	3.60±0.0500 ^a
Heart	TBARS	0.62±0.0007	0.92±0.0011*	0.64±0.0600 ^ε	0.73±0.0100 ^a
	GSH	124.60±6.0000	83.60±2.5000*	127.30±11.0000 ^ε	102.60±1.7000 ^a
	GPX	3.40±0.0800	2.20±0.0400*	3.20±0.0200 ^ε	4.10±0.0900 ^a
	Catalase	0.50±0.0070	0.20±0.0080*	0.50±0.0100 ^ε	0.50±0.0500 ^a

Values are Mean±SD. n=6. Statistical difference is calculated in one-way ANOVA method (least significant difference). *Statistical difference between Groups 1 and 2 ($P<0.05$). ^ε – No significant difference between Group 1 versus Group 3. ^aSignificant difference ($P<0.05$) between Group 1 versus Group 4. One nMol of MDA/mg of protein, 2 µg of GSH/mg of protein, 3 µg of GSH used/min/mg of protein, 4 µMol of H₂O₂ used/min/mg of protein. TBARS: "Thiobarbituric acid reactive substance," GSH: "Reduced glutathione," GPX: Glutathione peroxidase

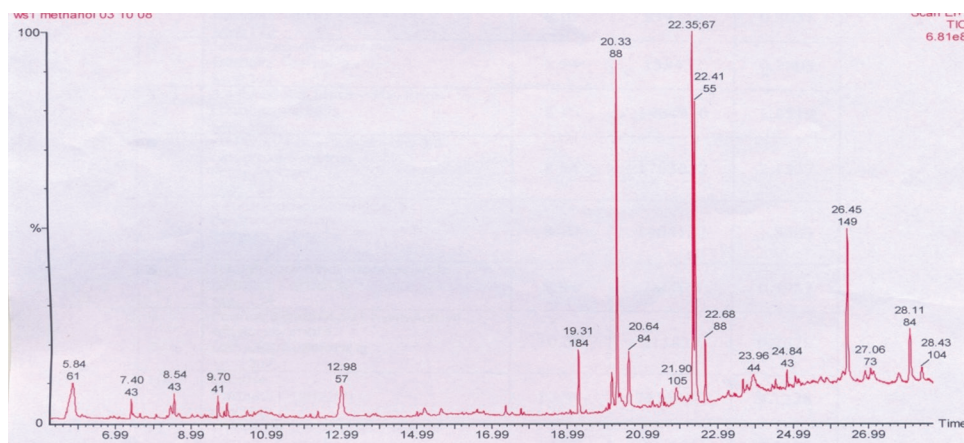


Fig. 1. Chromatogram gas chromatography–mass spectrometer of the chemical compounds of WS extract.

for the present research. The main purpose of selecting this model is to evaluate the myocardial membrane stabilizing effect through antioxidant and hypolipidemic activity of WS's 70% ethanolic extract. Minihane (Minihane et al., 2005) has also suggested that isoproterenol-induced cardiotoxicity is a widely accepted non-invasive model.

The primary purpose of selecting this model is to evaluate the myocardial membrane-stabilizing effect through antioxidant and hypolipidemic activity of WS's 70% ethanolic extract. Isoproterenol-induced cardiotoxicity has been suggested as it is a widely accepted non-invasive mode (Arnaldo et al., 2004). The modulation of oxidative stress by WS root powder has been reported earlier (Saleem et al.,

2020). Isoproterenol causes ischemia or oxidative stress but also causes positive inotropic and chronotropic effects on the heart (Fontana et al., 2007). These abnormal conditions lead to the damage of the heart. The deterioration in the heart tissue results in the release of cardiac markers and lipid accumulation in the myocardium (Subasini et al., 2009a). The myocardial membrane can be protected from damage induced by isoproterenol's hazardous nature by a medication having antioxidant action.

A drug that can act as an antioxidant and as a receptor antagonist can prevent the binding of isoproterenol with its receptor and thereby protect the heart from the toxic effect of isoproterenol. Reactive oxygen species are produced

TABLE V
GAS CHROMATOGRAPHY–MASS SPECTROMETER PEAK TABLE OF *WITHANIA SOMNIFERA*

Signal no.	Peak name	Retention time	% peak area
1	Glycerine	5.84	12.9203
2	2-Pyrazolin-5-one, 1,3,4, trimethyl-	7.40	2.0233
3	Phenylethyl alcohol	8.05	0.3031
4	Tetrahydro-4H-pyran-4-ol	8.34	0.2003
5	3,4-Furandiyl, tetrahydro-trans-	8.43	1.8519
6	4H-pyran-4-one, 2,3-Dihydro-3,5-	8.54	2.1537
7	2-Furancarboxaldehyde, 5-(Hydroxymethyl)-	9.70	1.8509
8	1,2,3-Propanetriol, monoacetate	9.94	0.9937
9	Phenol, 3-methyl-5-(1-methylethyl)-ethyl carbamate	10.66	0.2678
10	Sucrose	12.98	9.1228
11	1H-Pyrrole, 2,5-Dihydro-1-nitroso-	13.90	0.5008
12	3-Deoxy-d-mannoic-lactone	15.19	1.6182
13	Phenol-2,4,5-trimethyl-	17.37	0.9368
14	Benzidine, N-Vanilylidene-	19.31	5.1915
15	[1,1'-Bicyclopentyl]-2-one	20.19	3.6974
16	Hexadecanoic acid ethyl ester	20.33	24.2788
17	2-n-Hexylcyclopentanone	20.64	0.0884
18	Octadecanoic acid ethyl ester	21.53	1.4221
19	9,12-Octadecadienoic acid-ethyl ester	22.35	23.5742
20	4H-1,2,4-Triazol-3-amine-4-Propyl-	28.11	7.0041

by a variety of mechanisms including xanthine oxidase, nicotinamide adenine dinucleotide phosphate oxidases, cytochrome P450, catecholamine auto-oxidation, and nitric oxide (NO) synthase uncoupling NO synthases. Isoproterenol (ISO), a synthetic catecholamine, undergoes oxidation and generates superoxide anion. The superoxide radical initiates the chain reactions and results in free radical intermediates and lipid peroxidation. Drugs with antioxidant qualities may supplement endogenous defense systems and lower both the initiation and propagation of the lipid peroxidation process (Thenmozhi and Subasini, 2016). The decreased TBARS and increased activity of antioxidants observed in the present study might be due to the presence of withanolides (Peer et al., 2008). According to Badavi et al., 2020, ISO causes myocardial infarction in experimental animals by acting on the sarcoplasmic membrane, stimulating adenylate cyclase, activating Na⁺ and Ca²⁺ channels, and causing myocardium cell death. The elevated level of marker enzymes in serum may be due to the harm induced by these impacts (Subasini, 2009a). CK level is increased significantly ($P < 0.05$) in serum of diseased rats against normal rats. The results are in line with an earlier reference (Vimal and Devaki, 2004). The decreased level of LDH in heart tissue of diseased rats is in line with a previous referred reference (Saravanan et al., 2011). The reduced activity of LDH prevents the conversion of lactate to pyruvate. As mentioned above, ischemia increases anaerobic glycolysis.

Similarly, the GPT level considerably reduces the cardiac tissue of diseased group of rats. In experimental inflammatory circumstances, Priscilla (Priscilla and Prince, 2009) discovered that enzymatic tissue activities, particularly aminotransferases, increased. The WS extract's

mechanism may work by lowering Ca²⁺ inflow and avoiding enzyme leakage by reducing myocardial damage. Still, the mechanism of the protective action of this extract should be evaluated. The decreased release of cardiac markers by WS pre-treatment might be due to various antioxidant activity exhibiting compounds like withanolides (Durg et al., 2015).

Ipseeta et al., 2004, have shown that the WS treatment can prevent the influx of calcium, negative inotropic and chronotropic activity. Moreover, Gupta (Gupta et al., 2004) has explained that ISO causes increased myocardial lipids. It raises LDL cholesterol levels in the blood, causing dangerous deposits to form in the arteries and so favoring coronary heart disease.

In addition, ISO promotes lipolysis in the myocardium (Jasemi et al., 2020, and Ipseeta et al., 2004). Lipolysis results in the release of a large amount of free fatty acids and TAG. The decrement of TAG observed in our present study reveals that WS extract is exhibiting membrane stabilizing activity (Subasini et al., 2009b). GC–MS analysis of methanol-soluble volatile compounds of WS's 70% ethanolic extract has indicated fatty acids such as octadecadienoic acid, methyl ester, and hexadecadienoic acid ethyl ester. The hexadecadienoic acid-ethyl ester with a peak area of 24.27% is present in higher concentrations. The cardioprotective activity of this compound has not been reported earlier. The observed cardioprotective activity in the present study might be due to the presence of octadecadienoic acid, methyl ester, hexadecadienoic acid ethyl ester, and hexadecadienoic acid-ethyl ester. The action might be due to the synergistic action of all the mentioned above compounds. The extract contains sucrose and sucrose administration can induce lipid peroxidation and decrease Cu-Zn SOD (Jerome et al., 2002). The observed antioxidant activity might be due to the presence of other compounds like fatty acids, which are present in higher concentrations (peak area = 23.57%) than that of sucrose (peak area = 9.12%) (Subasini et al., 2007).

V. CONCLUSION

In Triton WR 1339 generated hyperlipidemic rats, WS has hypolipidemic action by lowering cholesterol and LDL levels. Similarly, WS extract administration decreases cardiotoxicity in isoproterenol-induced cardiotoxic rats through boosting antioxidant levels. Increased antioxidants also reduce TBARS release and cardiac marker release from cardiac tissue. Moreover, the WS extract also reduces the level of TAG in the plasma of isoproterenol administered in rats. The presence of fatty acids detected by the GC–MS spectrum is responsible for the extract's hypolipidemic, cardioprotective, and antioxidant activities.

REFERENCES

- Adams, J.D., Yang, J., Mishra, L.C. and Singh, B.B., 2002. Effects of Ashwagandha in a rat model of stroke. *Alternative Therapies in Health and Medicine*, 8, pp.18-19.
- Aebi, H., 1974. Catalase. In: Bergmayer, H.E., (Ed.), *Methods of Enzymatic Analysis*. 2nd ed. Verlag Chemie/Academic Press Inc., Weinheim/NewYork.

pp.673-680.

Arnaldo, P., Silvio, T., Livio, T., Sergio, B., Edgardo, B. and Roberto, A., 2004. Isoproterenol-induced myocardial infarction in rabbits protection by propranolol or labetalol: A proposed non-invasive procedure. *European Journal of Pharmaceutical Sciences*, 23(3), pp.277-285.

Badavi, M., Mard, S.A., Dianat, M. and Dashtbozorgi, N., 2020. Crocin attenuates oxidative stress and inflammation in myocardial infarction induced by isoprenaline via PPAR γ activation in diabetic rats. *Journal of Diabetes and Metabolic Disorders*, 19(2), pp.1517-1525.

Boehm, E., Ventura-Clapier, R., Mateo, P., Lechene, P. and Veksler, V., 2000. Glycolysis supports calcium uptake by the sarcoplasmic reticulum in skinned ventricular fibres of mice deficient in mitochondrial and cytosolic creatine kinase. *Journal of Molecular and Cellular Cardiology*, 32, pp.891-902.

Dar, P.A., Singh, L.R., Kamal, M.A. and Dar, T.A., 2016. Unique medicinal properties of *Withania somnifera*: Phytochemical constituents and protein component. *Current Pharmaceutical Design*, 22(5), pp.535-540.

Durg, S., Dhadde, S.B., Vandal, R., Shivakumar, B.S. and Charan, C.S., 2015. *Withania somnifera* (Ashwagandha) in neurobehavioural disorders induced by brain oxidative stress in rodents: A systematic review and meta-analysis. *Journal of Pharmacy and Pharmacology*, 67(7), pp.879-899.

Fontana, M., Olschewski, H., Olschewski, A. and Schlüter, K.D., 2007. Treprostinil potentiates the positive inotropic effect of catecholamines in adult rat ventricular cardiomyocytes. *British Journal of Pharmacology*, 151, pp.779-786.

Foster, L.B. and Dunn, R.T., 1973. Standard reagents for determination of serum triglycerides by colorimetric Hantzsch condensation method. *Clinical Chemistry*, 19, pp.338-340.

Friedewald, W.T., Levy, R.I. and Fredrickson, D., 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical Chemistry*, 8, pp.499-502.

Gupta, S.K., Mohanty, I., Talwar, K.K., Dinda, A., Joshi, S., Bansal, P., Saxena, A. and Arya, D.S., 2004. Cardioprotection from ischemia and reperfusion injury by *Withania somnifera*: A hemodynamic, biochemical and histopathological assessment. *Mol Cell Biochem.*, 260 (1-2), pp.39-47. doi: 10.1023/b: mcbi.0000026051.16803.03.

Ipseeta, M., Dharamvir, S.A., Amit, D., Keval, K.T., Sujata, J. and Suresh, K.G., 2004. Mechanisms of cardioprotective effect of *Withania somnifera* in experimentally induced myocardial infarction. *Basic and Clinical Pharmacology and Toxicology*, 94(4), pp.184-190.

Jasemi, S.V., Khazaei, H., Aneva, I.Y., Farzaei, M.H. and Echeverria, J., 2020. Medicinal plants and phytochemicals for the treatment of pulmonary hypertension. *Frontiers in Pharmacology*, 11, pp.145-166.

Jerome, B., Wioletta, Z., Edmond, R., Yves, R. and Andrzej, M., 2002. Rats fed a high sucrose diet have altered heart antioxidant enzyme activity and gene expression. *Life Science*, 71(11), pp.1303-1312.

King, J., 1959. Colorimetric determination of serum lactate dehydrogenase. *The Journal of Medical Laboratory Technology.*, 16, pp.265-269.

Kumari, M., Gupta, R.P., Lather, D. and Bagri, P., 2020. Ameliorating effect of *Withania somnifera* root extract in *Escherichia coli*-infected broilers. *Poultry Science*, 99(4), pp.1875-1887.

Majithiya, J.B., Parmar, A.N. and Balaraman, R., 2004. Effect of curcumin on triton WR 1339 induced hypercholesterolemia in mice. *Indian Journal of Pharmacology*, 36(6), pp.382-383.

Minihane, A.M., Brady, L.M., Lovegrove, S.S., Lesauvage, S.V., Williams, C.M. and Lovegrove, J.A., 2005. Lack of effect of dietary n-6:n-3 PUFA ratio on plasmalipids and markers of insulin responses in Indian Asians living in the UK. *European Journal of Nutrition*, 44(1), pp.26-32.

Mohun, A.F. and Cook, I.J.Y., 1957. Simple method of measuring serum levels of glutamate oxaloacetate and glutamate pyruvate transaminases. *Journal of Clinical Pathology*, 10, pp.374-399.

Moron, M.S., Depierre, J.W. and Mannervik, B., 1979. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. *Biochimica et Biophysica Acta*, 528, pp.67-78.

Nishant, P.V., Narasimhacharya, A., 2006. Hypocholesteremic and antioxidant effects of *Withania somnifera* (Dunal) in hypercholesteremic rats. *Phytomedicine*, 14(2-3), pp.136-142.

Ohkawa, H., Ohishi, N. and Yagi, K., 2004. Assay of lipid peroxide in animal tissue by thiobarbituric acid reaction. *Annals of Clinical Biochemistry*, 95(2), pp.351-358.

Paglia, D.E. and Valentine, W.N., 1967. Studies on the quantitative and qualitative characterization of erythrocyte peroxidase. *Journal of Laboratory and Clinical Medicine*, 2, pp.158.

Peer, P.A., Trivedi, P.C., Nigade, P.B., Ghaisas, M.M. and Deshpande, A.D., 2008. Cardioprotective effect of *Azadirachta indica* A. Juss. on isoprenaline induced myocardial infarction in rats. *International Journal of Cardiology*, 126(1), pp.123-126.

Priscilla, D.H. and Prince, P.S., 2009. Cardioprotective effect of gallic acid on cardiac troponin-T, cardiac marker enzymes, lipid peroxidation products and antioxidants in experimentally induced myocardial infarction in Wistar rats. *Chemico-Biological Interactions*, 179(2-3), pp.118-24.

Richmond, W., 1973. Preparation and properties of a cholesterol oxidase from *Nocardia* sp. and its application to the enzymatic assay of total cholesterol in serum. *Clinical Chemistry*, 19, pp.1350-1356.

Saggam, A., Lingaokar, K., Borse, S., Chavan-Gautam, P., Dixit, S., Tillu, G. and Patwardhan, B., 2021. *Withania somnifera* (L.) Dunal: Opportunity for clinical repurposing in COVID-19 management. *Frontiers in Pharmacology*, 12, pp.1-18.

Saleem, S., Muhammad, G., Hussain, M.A., Altaf, M. and Bukhari, S.N.A., 2020. *Withania somnifera* L.: Insights into the phytochemical profile, therapeutic potential, clinical trials, and future prospective. *The Iranian Journal of Basic Medical Sciences*, 23(12), pp.1501-1526.

Saravanan, S., Ramachandran, S., SujaRajapandian, Subasini, U., Victor R.G. and Dubey, G.P., 2011. Anti-atherogenic activity of ethanolic fraction of *Terminalia arjuna* bark on hypercholesterolemic rabbits. *Evidence-Based Complementary and Alternative Medicine.*, 2, pp.1-8.

Subasini, U. and Javed, A., 2019. Phytochemical analysis of different fractions of *Terminalia arjuna* bark by GC-MS. *International Research Journal of Pharmacy*, 10(1), pp.42-48.

Subasini, U., 2019. Novel biomarkers of atherogenic diet induced dyslipidemia and metabolic syndrome suppressed by *Terminalia arjuna*. *International Journal of Pharmaceutical Sciences and Research*, 10(5), pp.2528-2536.

Subasini, U., Mohamed, M., Shabi, G.K., Dhevi, R., Ramakrishnan, N., Victor, G., Rajamanickam, G.P. and Dubey, G.P., 2009. Phytochemical evaluation with hypoglycaemic and antioxidant activity of *Tribulus terrestris* Linn. *International Journal of Biomedicine*, 29(2), pp.121-127.

Subasini, U., Rajamanickam, G.V., Dubey, G.P., Prabu, P.C., Sahayam, C.S., Mohammed, S.M., Gayathri, K., Aruna, A., 2007. Hydroalcoholic extract of *Terminalia arjuna*: A potential hepatoprotective herb. *Journal of Biological Sciences*, 2, pp.255-262.

Subasini, U., Sundaraganapathy, R., Narmadha, M.P., Victor, G., Rajamanickam, G.V. and Dubey, G.P., 2009. Cardio-protective effect of polyherbal formulation in isoproterenol induced cardio toxicity. *International Journal of Pharma Research*, 2(3), pp.75-82.

Subasini, U., Thenmozhi, S., Venkateswaran, V., Pavani, P., Sumeet, D. and Victor, R.G., 2014. Phytochemical analysis and anti hyperlipidemic activity of *Nelumbo nucifera* in male wistar rats. *International Journal of Pharmacy Teaching and Practices*, 5(1), pp.935-940.

Thenmozhi, S. and Subasini, U., 2016. Isolation, characterization and *in-vitro* cytotoxic study of vitexin from *Vitex pinnata* Linn. Leaves. *International Journal of Research in Pharmacology and Pharmacotherapeutics*, 1, pp.84-89.

Vimal, V. and Devaki, T., 2004. Linear furanocoumarin protects rat myocardium against lipid peroxidation and membrane damage during experimental myocardial injury. *Biomedicine and Pharmacotherapy*, 58, pp.393-400.

Warnick, G.R., Bederson, J. and Albers, J.J., 1992. Dextran-sulfate-Mg²⁺ precipitation procedure for quantitation of high density lipoprotein cholesterol.

Clinical Chemistry, 28, pp.1379-1388.

Youssef, S., Stüve, O., Patarroyo, J.C., Ruiz, P.J., Radosevich, J.L., Hur EM, B.M., Mitchell, D.J., Sobel, R.A., Steinman, L. and Zamvil, S.S., 2002. The HMG-CoA reductase inhibitor, atorvastatin, promotes a Th2 bias and reverses paralysis in central nervous system autoimmune disease. *Nature*, 420(6911), pp.78-84.