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ANTI-HYPERLIPIDEMIC AND ANTIOXIDANT CAPACITY OF ACTIVE EXTRACTS OF *FRAGARIA NUBICOLA* IN HIGH FAT DIET FED HYPERLIPIDEMIC RATS

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ABSTRACT: Hyperlipidemia is a major risk factor for CHD, atherosclerosis and diabetes. Present study has been conducted to evaluate the antioxidant and anti hyperlipidemic activity of *Fragaria nubicola*. All *in-vitro* antioxidant activities were concentration dependent, which were compared with standard antioxidants BHT and Vitamin C. At concentration of 700 µg/ml the DPPH radical scavenging activity of methanol extract was more than that of aqueous and ethyl actetate extracts. The highest phenolic content was observed in methanolic extract followed by aqueous and ethyl acetate. Superoxide and hydroxyl radical scavenging activity of extracts increased in dose dependent manner with highest in methanolic extract. To evaluate hyperlipidemic activity, 35 rats weighing 80-100gm were divided into 7 groups with five in each group and were fed with high fat diet for one month. Group I contained normal receiving water only, group II Hyper-lipidemic receiving high fat diet, group III Atorvastatin (10 mg/kg), group IV and group V receiving methanolic extract of 50 and 100 mg/kg, group VI and VII receiving aqueous extract of 50 and 100 mg/kg body weight. There was a significant reduction in total cholesterol, triglyceride, LDL and increase in HDL levels in all the groups with extracts. The lipid lowering capacity of methanol extracts was more than that of aqueous extracts. The methanolic extract (100 mg/kg) showed a highly significant reduction in lipids along with the drug. The findings of present study suggest that the methanolic extract possess a good amount of antioxidant and anti hyperlipidemic activity and could have great importance as a therapeutic agent in future.

INTRODUCTION: Oxidative stress occurs when there is an imbalance between the production and quenching of free radicals from oxygen species. These reactive oxygen species (ROS) play a role in many chronic diseases including mitochondrial diseases [1].

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The partially reduced metabolites of oxygen and nitrogen, commonly referred to as free radicals are highly toxic and reactive. They have been postulated to be increased in majority of diseases like aging, atherosclerosis and cancer [2].

Reactive oxygen species (ROS), which consist of different free radicals such as hydroxyl (OH^-), superoxide (O_2^-), peroxyl (RO_2^-), lipid peroxyl (ROO^-) radicals and non free radicals such as hydrogen peroxide (H_2O_2), singlet oxygen (O_2^{-1}), ozone (O_3) and Lipid peroxide (LOOH) are different forms of activated oxygen. Reactive oxygen species are formed during normal cellular

metabolism but when present in high concentration they become toxic. Oxidative stress and ROS mediated cell damage are associated with the onset of many diseases such as cancer, rheumatoid arthritis, cirrhosis, atherosclerosis and degenerative processes associated with aging. Oxidative damage can cause base degradation, DNA fragmentation and cross-linking of proteins and disrupt the normal functioning of these molecules. Scavenging of reactive oxygen species (ROS) is important in preventing potential damage to cellular components such as DNA, proteins and lipids.

Antioxidants are the substances that are present in low concentration in the human body and delay or reduce the oxidation of the substrate. Humans have developed highly complex antioxidant systems (enzymatic and non-enzymatic), which work synergistically, and together with each other to protect the cells and organ systems of the body against free radical damage. When the normal levels of antioxidant defense mechanism of the human body is not sufficient for the eradication of free radical induced injury, administration of antioxidant will have a protective role to play [3].

Hyperlipidemia is a metabolic condition in which there is an increase in lipids and lipoproteins and is a major cause of atherosclerosis and the atherosclerosis associated conditions, such as coronary heart disease, hypertension, obesity and diabetes mellitus (Type -II) [4]. High levels of total cholesterol TC, low levels of high density lipoproteins HDL, high level of low density lipoproteins LDL and high levels of TG are major cause of increased atherogenic risk; both genetic disorders and sedentary lifestyle, diet high in calories, saturated fat and cholesterol contribute to hyperlipidemia seen in developed countries around the world [5]. It is evident from epidemiological studies that there is a strong relationship between atherosclerosis and elevated levels of plasma lipid. Increased plasma lipid levels are known to cause hyper-lipidemia which is core in initiation and progression of atherosclerosis impasse [6].

In recent years, there has been an increase of interest in the use of medicinal plants to treat various oxidative stress related ailments because herbal medicines have been reported as safe, easily available, economically effective and without any adverse side effects especially when compared with other synthetic drugs [7]. Plants contain many free radical scavenging molecules such as phenolic compounds, nitrogen compounds, Vitamins, flavonoids, terpenoids, *etc.* which help in defense against the production of free radicals. These plants produce some chemical substances which generate a specific physiological action on the human body.

Fragaria nubicola is a perennial herbaceous plant growing prostrate on ground. Fragaria is a genus of flowering plants in the rose family, Rosaceae, commonly known as wild strawberries. Its rhizome is traditionally used to cure inflammatory disorders and contains ellagic acid, acid-glycoside and various other glycosides. The fruits possess potential to have anti-inflammatory antioxidant and anti-neurodegenerative properties [8].

MATERIALS AND METHODS:

Chemicals: Chemicals used in this study were of analytical grade and of highest purity procured from standard commercial sources in India. Atorvastatin was obtained from market pharmacy. Diagnostic kits for the estimation of TC, triglyceride and HDL-C were obtained from ERBA.

Chloroform, Ferric chloride, TCA, Phosphate buffer, Gallic acid, Ferric nitrate, Cholesterol, Glacial acetic acid, Folin reagent, Na_2CO_3, H_2O_2, Picric acid, Ammonia, Hydrochloric acid, Lead acetate, EDTA, BHT, Riboflavin, Ascorbic acid and Agarose gel were purchased from (SRL). DPPH NBT, Sucrose, Metionine, Cholic acid and calf thymus DNA were purchased from (HIMEDIA).

Plant Collection and Identification: The fresh whole plant was collected from different regions of Kashmir valley in month of July and August. The plant was identified and authenticated by the courtesy of Centre of Plant Taxonomy, Department of Botany University of Kashmir. The specimen of the plant was retained in the herbarium with voucher specimen No 2063- KASH.

Preparation of Extracts: The whole plant material was shade dried under room temperature at 30 ± 2°C. The dried plant material was grinded into powder using mortar and pestle and sieved through a 0.3 mm aperture size sieve. The powder obtained was successively extracted in hexane, ethyl acetate, methanol and water by using soxhlet extractor (60-

80°C). The extraction of the plant was done according to the polarity of the solvents used in order of increasing polarity. The ethyl acetate, methanolic and aqueous extracts were then concentrated with the help of rotary evaporator under reduced pressure and the solid extracts were stored in refrigerator at 4 °C for further use.

Phytochemical Analysis: The conventional chemical tests were carried out for the extracts of different solvents to identify the presence of various chemical constituents. Phytochemicals were detected by the method of Harborne with little modifications.

DPPH Radical Scavenging Assay: The free radical scavenging activity of the extracts was determined by using DPPH method [9]. The DPPH radical solution was prepared in methanol. Various concentrations of plant extract (100-700 µg/mL) were added to 1ml of the 0.02% methanol solution of DPPH, and the mixture was vortexed vigorously. The tubes were then incubated at room temperature for 30 minutes in dark and the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. BHT was taken as known free radical scavenger. Percentage inhibition activity was calculated by

$$\% \text{ inhibition} = (A_0 - A_1) / A_0 \times 100$$

Where A_0 denotes the absorbance of the control and A_1 denotes absorbance in the presence of plant extract/known antioxidant.

Reducing Power: The estimation of reducing power was carried out according to the method described earlier with some modifications. Different concentrations of the plant extract were mixed with 2.5 mL of 0.2M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium hexacyanoferrate II. The mixture was incubated at 50^{O}C for 20 minutes, 2.5 mL of 10 % TCA was added to the mixture and centrifuged at 3000 rpm for 10 minutes. The upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5mL, 0.1%), and the absorbance was measured at 700 nm. BHT (butylated hydroxyl toluene) was taken as the known standard. The generation of Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Increased absorbance of the reaction mixture indicates stronger reducing power.

Total Phenolics: Total phenolic content was analyzed using the Folin-Ciocalteu reagent method. Gallic acid was used as the standard and the total phenolic contents were expressed as mg gallic acid equivalents per gram of tested extracts. The plant extract concentrations of (100-700µg/mL) were added to 2.5ml Folin-Ciocalteu reagent (10%) followed by 2 ml of 2.5% Na_2CO_3 solution and this mixture was shaken vigorously. The mixture was incubated for 45 minutes at 45^{o}C. The absorbance was checked at 700nm. Total levels of phenolics were calculated (with reference to Gallic acid) by referring the absorbance of the experimental samples with the standard regression curve.

Superoxide Anion Radical Scavenging Property: The superoxide radical generated from the photo reduction of riboflavin was detected by NBT reduction. The estimation of superoxide anion radical scavenging property was carried out as described earlier with some modifications [10]. The reaction mixture contained 100µl riboflavin solution (0.12mM), 200µl EDTA solution (12mM), 200µl methanol and 100µl NBT (Nitro-blue tetrazolium) solution (1.5mM) and reaction mixture was diluted up to 3ml with phosphate buffer (50mM, pH 7.6). Different concentrations of extracts were added to reaction mixture in test tubes, and further diluted up to 3 ml with phosphate buffer. Ascorbic acid was taken as control. Absorbance was measured after 5 min. at 530nm. Percentage inhibition activity was calculated by

$$\% \text{ inhibition} = (A_0 - A_1) / A_0 \times 100$$

Where A_0 denotes the absorbance of the control and A_1 denotes absorbance in the presence of plant extract/known antioxidant.

Hydroxyl Radical Scavenging Property: Hydroxyl radical, generated from the Fe^{3+}-Ascorbate-H_2O_2 (Fenton reaction), was evaluated by degradation of deoxyribose that produced thiobarbituric acid reactive species (TBARS) [11]. The reaction mixture contained 25mM deoxyribose, 20mM Ferric nitrate, 100mM ascorbic acid, 30 mM H_2O_2 in 10mM KH_2PO_4 (pH 7.4), and various concentrations of plant extracts. The reaction mixture was incubated at 37^{o}C for 1h then 1mL of

1% thiobarbituric acid and 1mL of 10% trichloro acetic acid was added and mixture heated at 100 ^{O}C for 20 min and cooled under tap water. The absorbance was measured spectrophotometrically at 532 nm. The percentage inhibition of hydroxyl scavenging activity was calculated using the following formula,

$$\% \text{ inhibition} = (A_0 - A_1) / A_0 \times 100$$

Where A_0 denotes the absorbance of the control and A_1 denotes absorbance in the presence of plant extract/known antioxidant.

DNA Protection Assay: DNA damage protection potential of extracts was evaluated as described earlier with some modifications. Mixture of 5µl of plant extract (1mg/ml) and 15µl of calf thymus DNA was added to 5µl of Fenton's reagent (30mM H_2O_2, 100mM ascorbic acid and 20mM ferric nitrate). The final volume was made up to 25µl with sterile water followed by incubating for 30 min at 37°C. The DNA was analyzed on 1% agarose gel using ethidium bromide staining.

Animal Studies:

Animals: Healthy albino rats of either sex weighing about 80-100g were used during the study. Standard environmental conditions such as temperature ranging from 20 to 32°C, relative humidity (70%) and 12 hrs dark/light cycle were maintained in the animal house. The animals had access to food, water and *libitum*. All procedures were performed in accordance to CPCSEA guidelines after approval from the Institutional Animal and Ethics Committee (IAEC) of the Department of Pharmacy, University of Kashmir No. F (IAEC-Approval/KU/2015/12) Dated 21/08/2015.

Induction of Hyperlipidemia: Rats were divided into seven groups each containing six rats. Normal animal food pellets were grinded into fine powder in mixer grinder. Then a high fat diet consisting of cholesterol (10 %), cholic acid (1 %), methionine (0.8 %), coconut oil (25%), and sucrose (40 %) was added with standard pellet diet and given for 30 days. This dried powder was then mixed with same quantity of water every time to make small balls of feed and later this was stored in refrigerator at 2°C to 6°C. The feed for normal group was prepared similarly by grinding only the normal food pellets and then mixing with water without the other excipients.

Experimental Design:

Group I: Normal

Group II: High fat diet

Group III: High fat diet + Atorvastatin (10mg/kg)

Group IV: High fat diet + Methanolic extract (50mg/kg)

Group V: High fat diet + Methanolic extract (100mg/kg)

Group VI: High fat diet + Aqueous extract (50mg/kg)

Group VII: High fat diet + Aqueous extract (100mg/kg)

Lipid Profile: The blood was collected from retro-orbital plexus without the use of anticoagulant. The blood was allowed to stand for 10 minutes before being centrifuged at 3000 rpm for 10 min to obtain serum for analysis. The blood from all the groups were collected both after giving HFD for 30 days and extracts for 15 days. Total cholesterol (TC), triglycerides (TG), LDL-cholesterol and high density lipoprotein cholesterol (HDL-C) were measured using standard diagnostic kits supplied by ERBA by using the ERBA LisaScan EM Analyser.

Statistical Analysis: All the values were expressed as mean ± SD. The results were evaluated by using the SPSS (version 12.0) and one way ANOVA followed by Bonferroni t-test. $P<0.05$ was considered significant.

RESULTS: In this study *Fragaria nubicola* was screened for phytochemicals. The plant is very rich in different types of phytochemicals such as flavonoids, phenols, tannins and saponins. Alkaloids and phlobtannins were observed in methanolic extract only. Steroids and volatile oils were absent from all the four extracts.

Methanolic extract was rich in most of the phytochemicals. Aqueous and ethyl acetate extract contained some of the phytochemical constituents. Only three phytochemical constituents like saponins, flavonoids and phenols were found in hexane. The phytochemical screening of all the extracts is shown in **Table 1**.

TABLE 1: PHYTOCHEMICAL SCREENING OF DIFFERENT EXTRACTS OF *FRAGARIA NUBICOLA*

Phytochemical	Methanol	Aqueous	Ethyl Acetate	Hexane
Alkaloids	+	-	-	-
Terpenoids	+	+	-	-
Saponins	++	++	++	+
Flavonoids	++	++	+	-
Tannins	+	+	+	+
Steroids	-	-	-	-
Phenols	++	+	++	+
Anthraquinones	+	+	-	-
Cardiac Glycoside	+	+	-	-
Phlobtannins	+	-	+	-
Cardenoloids	+	-	-	-
Volatile Oils	-	-	-	-

+ (present)-(Absent)

DPPH Radical Scavenging Activity: The DPPH radical scavenging activity of the known antioxidant BHT at 700µg/ml was the highest (95.87 ± 2.15%) followed by methanol, aqueous and ethyl acetate extract having 87.17 ± 1.02 %, 61.45 ± 1.45% and 45.90 ± 2.12 %, respectively (**Fig. 1**). The percentage inhibition observed was in the following descending order BHT > methanolic extract > aqueous extract > ethyl acetate extract.

The results show that the DPPH radical scavenging ability (IC_{50} value) of methanolic extract *i.e.*, 386.92 ± 10.10µg/ml, is reasonably significant in comparison to that of the standard BHT (183.85 ± 3.88µg/ml), followed by those of the aqueous (484.02 ± 5.58µg/ml) and ethyl acetate (842.02 ± 17.87µg/ml) extracts. These results suggest that methanolic extract of *Fragaria nubicola* exhibits the highest activity.

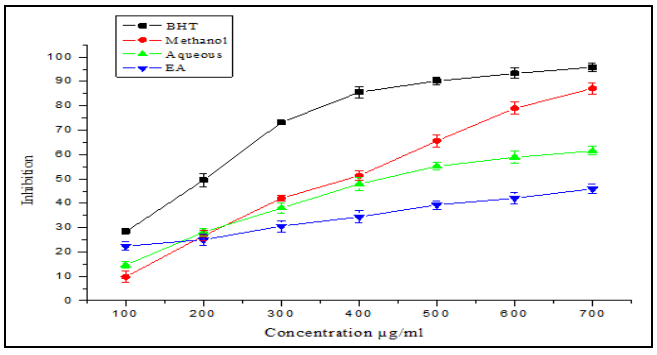

FIG. 1: DPPH RADICAL SCAVENGING ACTIVITY OF EXTRACTS

Hydroxyl Radical Scavenging Activity: At a concentration of 100µg/ml the scavenging activity of BHT was 90.32 ± 1.47 %, methanolic extract had 77.67 ± 0.92 %, aqueous extract had 57.02 ± 1.02 % and ethyl acetate had 59.68 ± 7.72 % (**Fig. 2**).

The percentage inhibition observed is in the following descending order BHT > methanolic extract > ethyl acetate extract > aqueous extract. The methanolic extract was found to be more effective in quenching the hydroxyl radicals produced in the reaction mixture.

Superoxide Radical Scavenging Activity: The superoxide radical scavenging activity of ascorbic acid, methanolic, aqueous and ethyl acetate extracts are shown in **Fig 3**. The extract and the standard demonstrate a dose response inhibition on super oxide anion radical. The percentage inhibition of superoxide generation by ascorbic acid and the extracts was 79.00 ± 2.95, 64.22 ± 2.54, 53.62 ± 3.76 and 42.92 ± 2.86 respectively at a higher concentration of 100µg/ml. The known antioxidant ascorbic acid exhibited an IC_{50} value of 49.95 ± 1.84µg/ml. Methanolic extract exhibited an IC_{50} value of 71.65 ± 1.12 µg/ml. The aqueous and ethyl acetate extracts show an IC_{50} value of 98.34 ± 2.45µg/ml and an IC_{50} value of 128.91 ± 0.95 µg/ml.

The methanolic extract was found to be an effective scavenger of super oxide radical generated by photo reduction of riboflavin.

Reducing Power Activity: Reducing power of all three extracts increased with increase in concentration. The aqueous extract showed more effective reductive ability when compared to that of methanolic and ethyl acetate extracts (**Fig. 4**). The known reducing agent taken here was BHT. For the measurement of the reductive ability, the Fe^{3+}-Fe^{2+} transformation was investigated in presence of the extract. Presence of reductants causes the reduction of the Fe^{3+} ferricyanide complex to the Fe^{2+} form.

At the highest concentration of plant extract 450 µg/ml BHT showed the reducing power of 1.387 and methanol extract showed 0.338. At the same concentration aqueous extract showed 0.447 and ethyl acetate had 0.246.

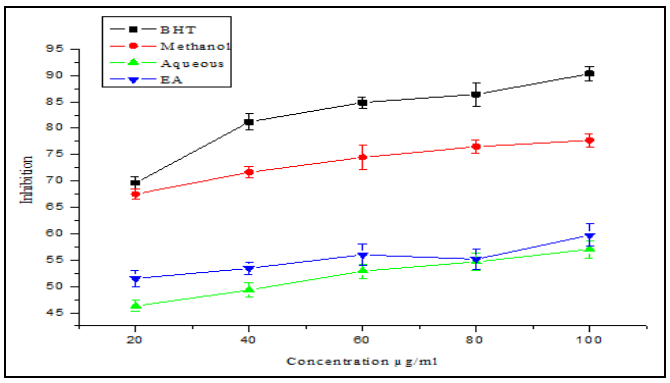

FIG. 2: HYDROXYL RADICAL SCAVENGING ACTIVITY

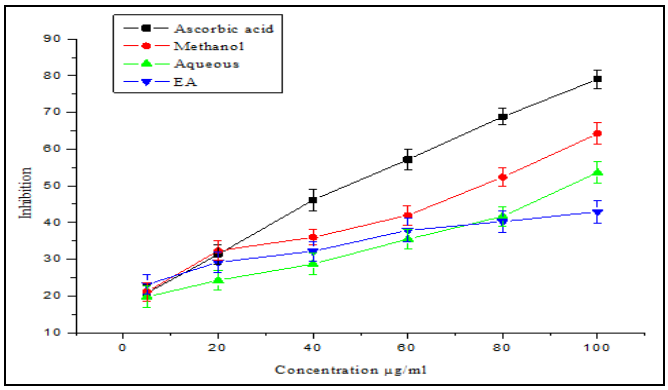

FIG. 3: SUPEROXIDE RADICAL SCAVENGING ACTIVITY

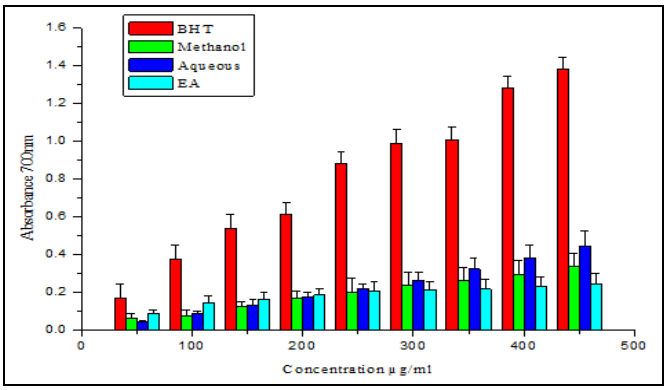

FIG. 4: REDUCING POWER ACTIVITY

Determination of Total Phenolic Content: Total phenolic content was determined by Folin Ciocalteau method and expressed as mg/1g of gallic acid equivalent. The phenol content of the methanolic, aqueous and ethyl acetate extract was found to be 843.68 ± 13.28 mg/g, 596.6 ± 8.54 mg/g and 411.1 ± 9.61 mg/g respectively. It is evident from the results that the phenolic content was found in considerable amount in all the extracts. Among the three extracts tested, aqueous extract exhibited high quantity of phenolic compounds compared to methanolic and ethyl acetate extract.

DNA Protection Assay: The DNA was fragmented using Fenton's reagent to check the ability of extracts in its repairing. Then the DNA was run on 1% agarose gel and viewed under UV light. The Calf thymus DNA was fragmented as shown in Fig 8 and the increasing concentration of plant extracts showed good protection of DNA against the hydroxyl radicals. The role of plant extracts in preventing DNA damage was assessed in this assay. The methanolic extract showed stronger protective effect against hydroxyl released from Fenton's reaction compared to aqueous and ethyl acetate extracts.

The protection effect of methanolic extract is shown in **Fig. 5**. The effect of aqueous extract is shown in **Fig. 6** and **Fig. 7** represents the effect of ethyl acetate extract.

Lipid Profile: All the groups were supplemented with high fat diet for 30 days and lipid profiling was done to check the levels of lipid content in the serum. Every group showed significant increase in serum total cholesterol, Triglyceride and LDL-C, but significant decrease in HDL-C level. The high fat diet was mixed with normal pellet diet along with water. This diet was given for 30 days in order to induce the hyperlipidemia. The blood was collected from all the groups. Lipid profiling of all groups were done to check the amount of lipids in the serum after giving high fat diet. The serum levels of TC, TG, LDL, HDL after supplementing HFD is shown in **Table 2**. They showed a good amount of lipids in their serum.

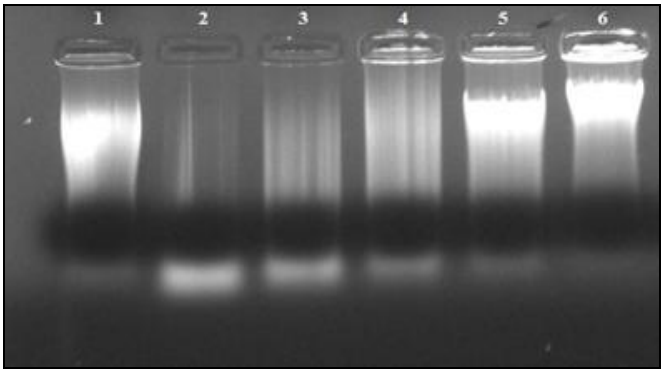

FIG. 5: AGAROSE GEL ELECTROPHORESIS OF METHANOLIC EXTRACT LANE 1 INTACT DNA, LANE 2 DNA WITH H_2O_2, LANE 3 EXTRACT 10 mg, LANE 4 EXTRACT 30 mg, LANE 5 EXTRACT 50 mg, LANE 6 EXTRACT 80 mg

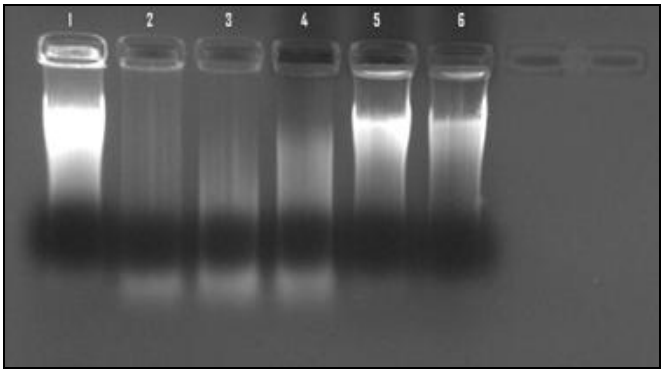

FIG. 6: AGAROSE GEL ELECTROPHORESIS OF AQUEOUS EXTRACT LANE 1 INTACT DNA, LANE 2 DNA WITH H_2O_2, LANE 3 EXTRACT 10 mg, LANE 4 EXTRACT 30 mg, LANE 5 EXTRACT 50 mg, LANE 6 EXTRACT 80 mg

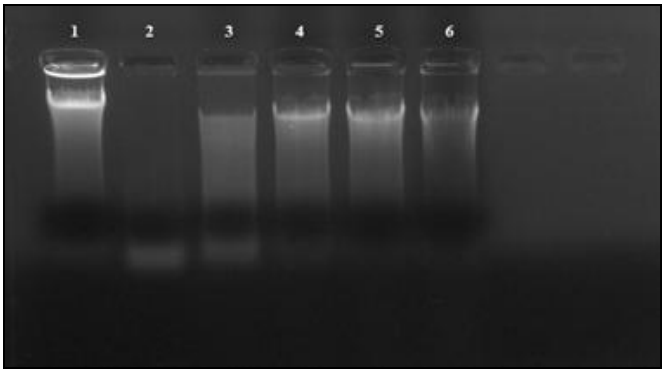

FIG. 7: AGAROSE GEL ELECTROPHORESIS OF ETHYL ACETATE EXTRACT LANE 1 INTACT DNA, LANE 2 DNA WITH H_2O_2, LANE 3 EXTRACT 10 mg, LANE 4 EXTRACT 30 mg, LANE 5 EXTRACT 50 mg, LANE 6 EXTRACT 80 mg

After confirming the good content of lipids in the serum the extracts were given to the specific groups. The extracts were given for 15 days along with high fat diet and the lipid profiling was done after the treatment with extracts. The methanolic and aqueous extract of concentration 50 mg/kg and 100 mg/kg was given to animals along with the standard drug atorvastatin 10 mg/kg. The standard drug atorvastatin (10 mg/kg) and the dose of methanolic extract (100 mg/kg) showed the maximum reduction in lipid levels ($p<0.01$) and increase in HDL-C ($p<0.01$). The methanolic extract of 50 mg/kg and both the aqueous extracts of (50 mg/kg and 100 mg/kg) showed a significant reduction in total cholesterol, TG and LDL ($p<0.05$) but considerable increase in HDL-C ($p<0.05$) when compared with control group. The effect on lipid profile by methanolic extracts and aqueous extracts are shown in **Table 3**.

TABLE 2: SERUM LIPID VALUES AFTER GIVING HIGH FAT DIET

S. no	Groups	Serum Lipid Parameters (mg/dl)			
		Total Cholesterol	Triglycerides	LDL	HDL
I	Normal control	85.22 ± 2.25	90.56 ± 1.02	42.22 ± 2.56	35.68 ± 2.14
II	Hyperlipidemic control	180.21 ± 8.25	175.58 ± 10.02	120.35 ± 9.95	21.20 ± 4.96
III	Atorvastatin (10 mg/kg)	184.55 ± 10.88	177.12 ± 6.55	108.88 ± 6.28	19.71 ± 2.26
IV	Meth. Ext (50 mg/kg)	181.36 ± 12.36	170.42 ± 8.33	110.65 ± 4.03	24.42 ± 5.07
V	Meth. Ext (100 mg/kg)	178.98 ± 8.15	168.84 ± 5.27	112.65 ± 5.52	22.69 ± 3.29
VI	Aq. Ext (50 mg/kg)	180.26 ± 9.21	175.96 ± 9.44	106.95 ± 3.32	20.22 ± 3.12
VII	Aq. Ext (100 mg/kg)	185.65 ± 10.85	165.54 ± 7.22	115.47 ± 6.78	18.78 ± 4.86

Each value represents the mean ± S.D. of 5 animals.

All the test groups were compared with control group and atorvastatin as shown in **Fig. 8**. The effect on lipid profile by methanolic extract 100 mg/kg was almost equal to standard drug atorvastatin 10 mg/kg (**Fig. 8**).

TABLE 3: SERUM LIPID VALUES AFTER TREATMENT WITH EXTRACTS

S. no	Groups	Serum Lipid Parameters (mg/dl)			
		Total Cholesterol	Triglycerides	LDL	HDL
I	Normal control	83.53 ± 6.23	89.69 ± 2.28	40.28 ± 3.14	36.54 ± 2.88
II	Hyperlipidemic control	185.41 ± 10.20	177.06 ± 6.23	118.81 ± 6.54	20.12 ± 2.11
III	Atorvastatin (10 mg/kg)	74.36 ± 8.66**	87.16 ± 8.94**	43.27 ± 4.98**	42.54 ± 2.22**
IV	Meth. Ext (50 mg/kg)	118.42 ± 5.95*	113.32 ± 6.33*	70.68 ± 8.23*	28.19 ± 3.91*
V	Meth. Ext (100 mg/kg)	92.28 ± 8.21**	105.24 ± 5.54**	52.31 ± 5.06**	37.08 ± 1.36**
VI	Aq. Ext (50 mg/kg)	142.34 ± 11.54*	128.01 ± 8.09*	76.54 ± 10.20*	23.65 ± 5.38
VII	Aq. Ext (100 mg/kg)	131.29 ± 9.23*	122.57 ± 8.31*	71.83 ± 8.16*	26.38 ± 4.82*

Each value represents the mean ± S.D. of 5 animals. *P<0.05 significant and **P<0.01 highly significant. LDL: low density lipoproteins, HDL: High density lipoproteins

Effect on Body Weight: The body weight of all the rats was checked regularly after every five days. It showed increase in all the groups. It increased from 80 - 100g to 300 - 330 g after giving the high fat diet for 30 days. The rats show gradual decrease in weight after treatment with plant extracts. The methanolic extract showed a significant decrease in weight as compared to aqueous extract. After giving the extracts for 15 days the body weight of the rats went back to 170 - 190 g as shown in **Fig. 9**.

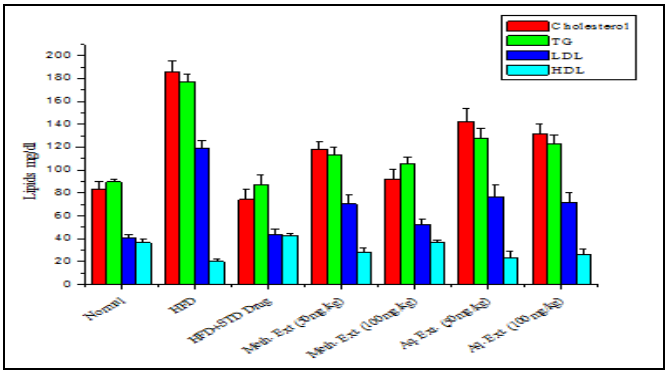

FIG. 8: EFFECT OF EXTRACTS OF *F. NUBICOLA* ON HIGH FAT DIET FED RATS IN COMPARISON WITH CONTROL GROUP

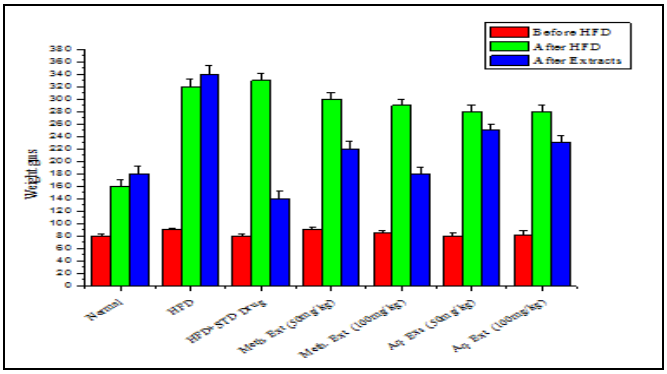

FIG. 9: EFFECT OF EXTRACTS OF *FRAGARIA NUBICOLA* ON BODY WEIGHT

DISCUSSION: The oxidative stress is the result of the free radicals which give rise to diseases like diabetes, cardiovascular diseases, cancer, cognitive *etc*. Oxidative stress arises when the production of reactive oxygen species overwhelms the intrinsic antioxidant defenses [12]. The increased level of oxidative stress is reflected by increased protein and DNA oxidation, enhanced lipid peroxidation, decreased level of cytochrome-c oxidase and advanced glycosylation end products. Lipid peroxidation can weaken cell membranes which causes ion imbalance and impairs the function of the cells [13].

When the number of free radicals increases to the point that they outnumber the antioxidants, they can attack the somatic cells and immune system [14]. Plants (fruits, vegetables, medicinal herbs, *etc*.) may contain a wide variety of free radical scavenging molecules, such as phenolic compounds, nitrogen compounds, alkaloids, vitamins, and some other endogenous metabolites, which are rich in antioxidant activity. These antioxidant compounds possess activities that help in protection against many diseases [15]. Hyperlipidemia, a disorder of lipid metabolism characterized by elevated levels of lipids circulating in the blood, has now become a global concern. It is considered as one of the five leading causes of death in the world today [16]. Hyperlipidemia is associated with the development of cardiovascular and metabolic syndrome diseases [17].

Hence it is essential to find an alternative to the management of hyperlipidemia. Hyperlipidemia promotes accumulation of oxidatively modified low density lipoproteins in the arterial wall, promoting endothelial dysfunction and development of atherosclerosis and congestive heart diseases. There are numerous herbal medicines exerting good hypolipidemic actions with fewer side effects

in Asian and African countries [18]. Plant materials have long been used as traditional medicines for the treatment of a wide variety of ailments and diseases. Various plants have been shown to lower plasma lipid levels and there are still many plants which need to be evaluated for treating different diseases [19, 20]. Plants are rich source of bioactive compounds and thus serve as a very important raw material for the drug development.

Medicinal plants are rich in various types of phytochemicals like alkaloids, steroids, flavonoids, anthraquinones, saponins, terpenoids, cardiac glycosides, tannins and cardenolides, *etc* [21]. *Fragaria nubicola* shows a rich phytochemical content and methanolic extract has the highest antioxidant activity. All the experimental groups showed increase in the body weight after giving high fat diet for 30 days. The methanolic and aqueous extracts showed decrease in the body weight and it is evident by comparing with the control group. The possible mechanisms for the *Fragaria nubicola* extracts to lower hyperlipidemia could be due to the increase of HDL-C, which is attributed to the mobilization of cholesterol from peripheral cells to the liver, decreasing the LDL oxidation or cholesterol inhibition.

The antioxidant property of the extracts could have many functions in the prevention of these diseases. The LDL oxidation is the main reason in the progression of atherosclerosis. So the administration of compounds which lower the LDL oxidation could play an important role in the prevention of this disease. It is reported that, the use of strawberries in the prevention of cancer, cardiovascular and other chronic diseases is due to its antioxidant activity [22].

CONCLUSION: This study evaluates the antioxidant and anti hyperlipidemic activity of strawberry plant *Fragaria nubicola.* It is concluded from the study that the methanolic extracts (50 mg/kg and 100 mg/kg) showed a significant activity in lowering the lipids. The methanolic extract (100mg/kg) showed more activity as compared to other extracts along with the standard drug. The aqueous extracts also have a fine anti hyperlipidemic potential. This can be used in future to test its potential against cardiovascular diseases especially in humans. The study helps in understanding the role of *Fragaria nubicola* as an antioxidant and anti hyperlipidemic agent. There is still a need for further clinical studies to isolate the specific component which has the anti-hyperlipidemic activity and to check its role in treating the disease in humans.

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