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In-Vitro Immunosupressant and Anti Dyslipidemic Activity of Fistein in Homocystein Induced in Rats

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ABSTRACT

Purpose: The study was aimed to investigate the immunospressant, antioxidant and anti dyslipidemic activity of *Fistein* in homocystein induced rats. **Methods**: Estimation of lymphocytes, monocytes and netrophils by flowcytometer, oxidative stress and antidyslipidemic activity were examined in homocystein induced groups and *fistein* pre-treated the rats for 28 days. **Results**: The *fistein* significantly (P<0.05) reduced the white blood cells of lymphocyte, monocytes and netrophils, oxidative stress and elevated dyslipidemea levels in homocystein induced negative control group, but increased the serum HDL status in all the fistein treatment groups, when compared with induced group. **Conclusion**: The present investigation suggests that fistein inhibits the elevated lipid profile levels, immune cells and oxidative stress.

INTRODUCTION

Plants producing numerous bioactive products among them the flavonoid plays a central role by their pharmacological properties for several diseases. Dietary flavonoids represent the principal anti-microbial and anti-inflammatory componenst provided by edible plants that have an impact on human health including cancer [1-5]. We have recently identified fisetin (3, 3', 4', 7-tetrahydroxyflavone), a flavonoid found in several fruits, vegetables, nuts and wine. Fisetin possesses anti-inflammatory activity [6, 7] and was found to be cytotoxic and antiangiogenic *in vitro* [8, 9]. Moreover the fisetein molecule bearing 4 hydroxyl substituents, including a catechol on the phenyl B ring, is therefore probably extensively metabolized *in vivo* and having potential biological activity. The fistein exhibited a wide variety of activities including neurotrophic, antioxidant, anti-inflammatory, and antiangiogenic effects [10, 11]. In the present study we determined the immunospressant, antioxidant and antidyslipidemic activity of *Fistein* in homocystein induced rats.

MATERIALS AND METHODS

Chemical agents: Fistein, DPPH purchased from Sigma Chemicals (St. Louis, MO USA). Flocytometry instrument from IICT Hyderabad, DMSO from Merck Pvt. Ltd. Hyderabad, Lipid profile kits procured from Excel diagnostics, Pvt. Ltd. Hyderabad.

Experimental design as follows that, the rats were randomly divided into 4 groups (n =6) as follows, Group I served as normal control, Group II served negative control (Homocystein 12mg/kg i.p), Group III treated with low dose Fisten (15 mg/kg per oral (*p.o*) and Group IV treated with high dose Fistein (30 mg/kg per oral (*p.o*).

Biochemical evaluation:

Blood samples were collected from the retro orbital vein puncture of rats in all the groups on 28^{th} day with Low dose & High doses (15 mg/kg & 30 mg/kg) of Fistein pretreatment groups and homocystein group, blood samples were centrifuged at 3000 rpm for 15 minutes and determined the antioxidant levels (12), Lipid profiles (13) and immunosuppressant levels (14).

Immunosuppressant activity (Whole blood method):

Collected 100 µl of whole blood for analysis; RBC lysing solution composition, briefly, 0.826 gm NH_4Cl, 0.119 gm $NaHCO_3$ and 20 µl of EDTA (0.5M) PH 8 and adjust PH 7.3

make up volume 100 ml has been used to not interfere RBC's with other cells in FACS analysis. This procedure allows different percentage populations of lymphocytes, monocytes and granulocytes by forward and side scatters. Incubate cells with RBC lysing buffer for 5 minutes and centrifuge at 1500 rpm for 3 minutes and repeat same for 2 times. Dilute the pellet in 200 µl 1x PBS buffer and load sample for analysis using with flow cytometer.

Total antioxidant (DPP- Method) activity: *In vitro* Antioxidant activity: DPPH free radical-scavenging activity: To determine the antioxidant activity a method based on the reduction of a methanolic solution of the coloured 1, 1-diphenyl-2- picryl-hydrazyl (DPPH) was used. The methanolic solution of DPPH (0.1 mM, 1 ml) was incubated with 3 ml of different concentrations ranging from 100-300 µg/ml of fistein. Incubation was carried out at room temperature (25^0C) for 30 minutes, for each concentration, the assay was run in triplicate. At the end of the incubation period, the optical density of each sample was determined at 517 *nm*. Ascorbic acid solution was used as a standard. IC50 values (the concentration of substance that provides 50% inhibition) for both ascorbic acid and fistein were determined. The free radical scavenging activity of the tested sample was expressed as an inhibition percentage (IP).

% inhibition of DPPH (or) % DPPH Scavenged = {(ADPPH – Atest)/ ADPPH} X 100

Where, DPPH is the absorbance of the 0.1 mM of DPPH solution and A test is the absorbance in the presence of the fistein or ascorbic acid. IC50 value was determined from the standard graph obtained using standard ascorbic acid by using the "y = mx + c" formula from the slope of the graph (12)

Treatment of Homocystein: Homocystein was administered by injection (120 mg/kg *i. p)* prior to at least 16 hours fasting, after 28 days treatment of fisetein 15 mg/kg and 30mg/kg per oral (*p.o)*. The blood samples were collected and estimated the differential leucocyte count (DLC), free radical scavenging activity and lipid profiles were measured.

Experimental animals: White male Wister rats weighing about 150-200 gm were purchased from the NIN Hyderabad., they kept under observation for about 1 week before the onset of the experiment to exclude any undercurrent infection. The chosen animals were housed in plastic aerated cages at normal atmospheric temperature (25±5°C) and normal 12 hours light/dark cycle. They had free access to water and standard diet of known composition *ad*

libitum. All animal procedures were in accordance with the recommendations of the ethical committee guidelines for the care and use of animals.

Statistical analysis

The data were represented as Mean ± SD statistical comparisons were made by one-way analysis of variance (ANOVA) and followed by Student-Neuman-Keuls test. Data were considered significant when *p* values were less than 0.05.

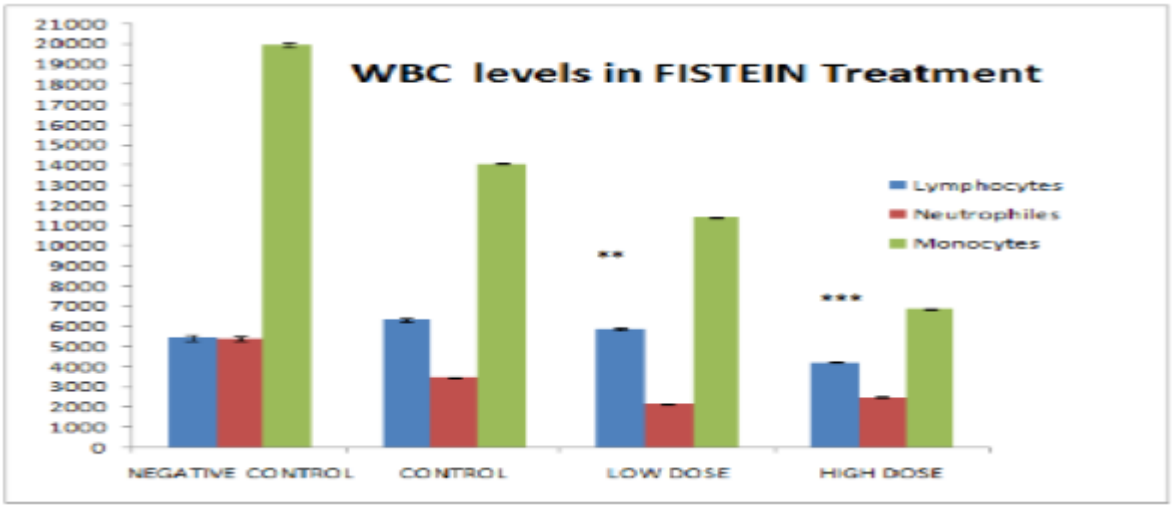
RESULTS

The effect of preteatment with Fistein at the doses of 15 mg/kg and 30 mg/kg on immune cells is that white blood cells (WBCs) levels in Homocystein induced results were obtained by Flocytometry detection, there is increased the WBC levels in Homocystein treated compared to normal control group and significantly ($p<0.005$) reduced the WBCs in Fisetin treatment groups and summarized the results in Fig1 & flowcytometry results were shown in figures 1a, 1b, 1c, 1d, respectively. Lipid profiles of LDL-cholesterol (LDL), Triglycerides (TG), total cholesterols levels were increased and decreased the HDL cholesterol in Homocystein treated group but in Fisetin pretreated groups significantly ($p<0.001$) reduced the Total cholesterol, LDL,VLDL, TG and increased the HDL cholesterol levels the results were represented in Table 1. *In-vitro* antioxidant levels decreased in Homocystein treated group but increased significantly ($p<0.001$) with Fistein and Ascorbic acid treated groups which were represented in Fig2. All the results were shown that increased the lymphocytes, oxidative burden and lipid contents concentration in Homocystein treated groups and the Fistein was significantly ($p < 0.001$) reduced the triglycerides, LDL-cholesterol, total cholesterol, oxidative burden and lymphocytes but increased HDL- cholesterol levels in pretreatment with low dose and high doses of Fistein.

Table No. 1: Lipid profile levels in normal, negative control and treatment groups

Parameters/ Groups	Normal	Homocystein	Fistein-I (15 mg/kg)	Fistein –II (30 mg/kg)
Cholesterol (mg/dl)	84.3±1.34	284.42±6.19	146.8±4.24 **	134.48±2.39***
Triglycerides (mg/dl)	67.76±2.3	188.19±3.33	122.58±3.67**	115.96±3.5 ***
HDL (mg/dl)	38.8±1.2	16.33±0.34	28.3±2.5 **	32.3±2.07 ***
LDL (mg/dl)	124.73±1.2	218.5±5.01	130.16±4.15**	123.98±2.87***
VLDL (mg/dl)	14.54±0.15	36.4±1.02	24.2±0.33 **	19.29±0.7 ***

(Data were mean ± SD; ** p<0.01, *** p<0.001 vs. diabetic and treatment groups).



(Data were mean ± SD; ** p<0.01, *** p<0.001 vs. Negative control and Treatment groups).

Figure No. 1: WBC levels in FISTEIN Treatment, Normal, & Negative control groups.

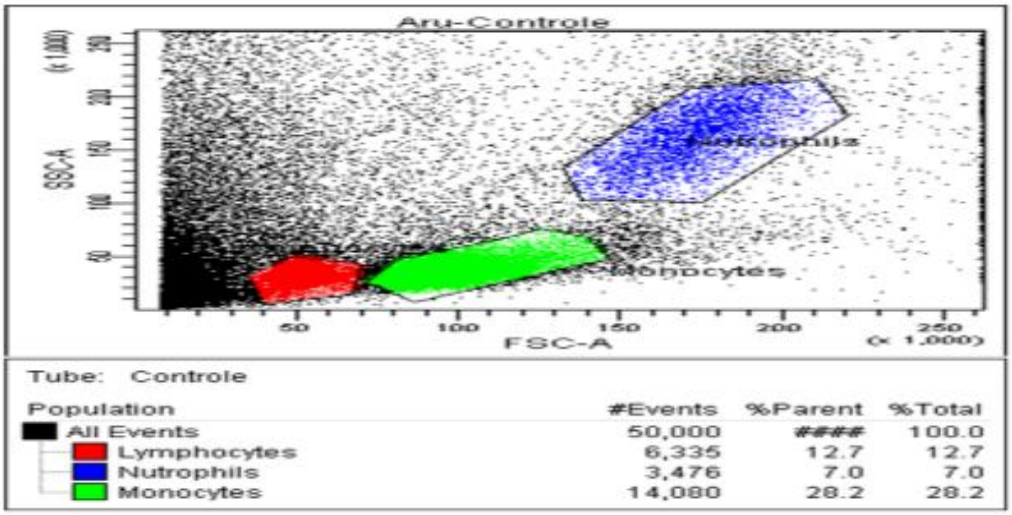


Figure No. 1a: Control

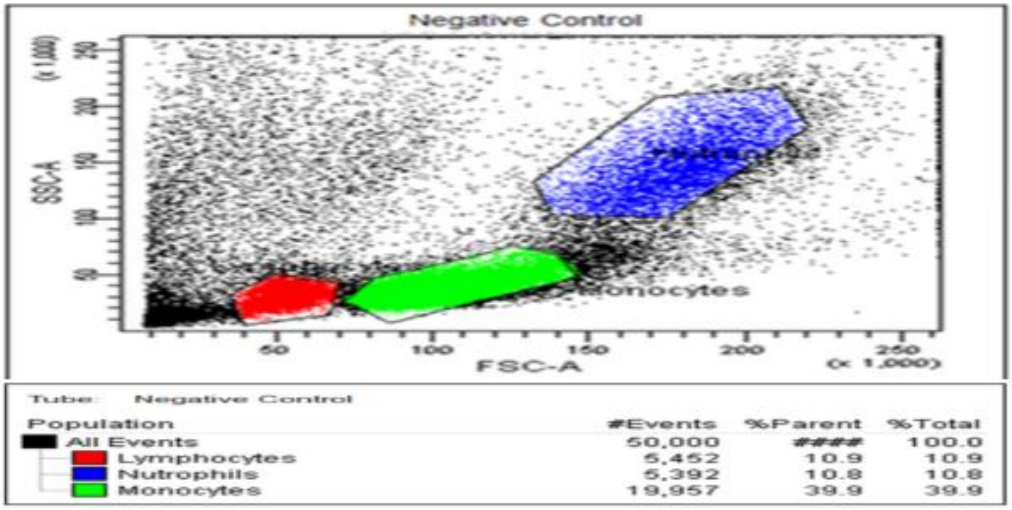


Figure No. 1b: Negative control

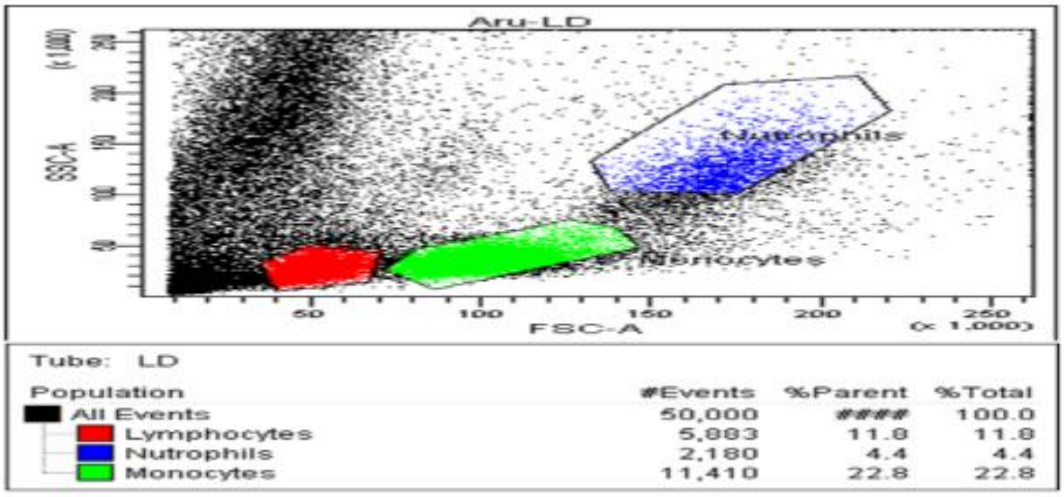


Figure No. 1c: Low dose

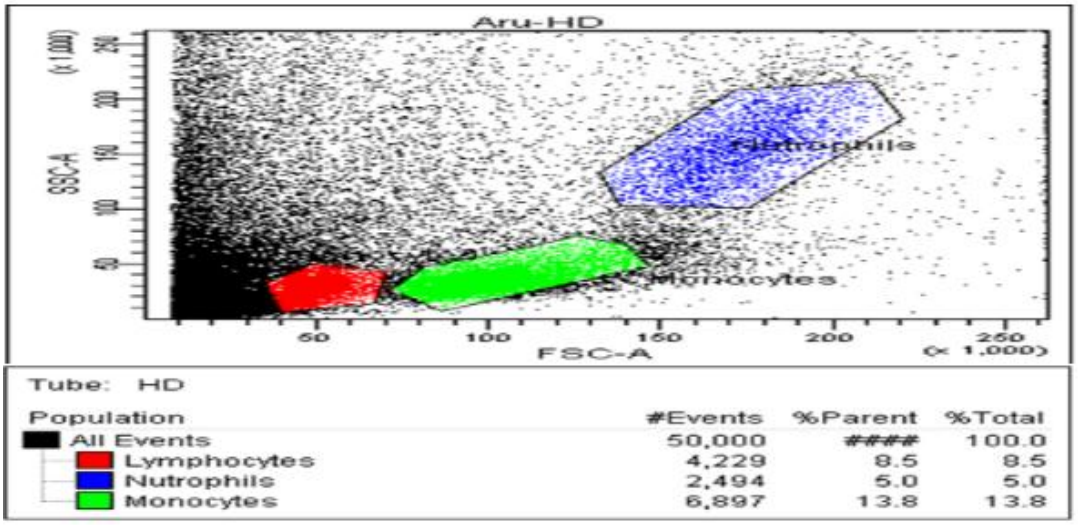


Figure No. 1d: High dose group

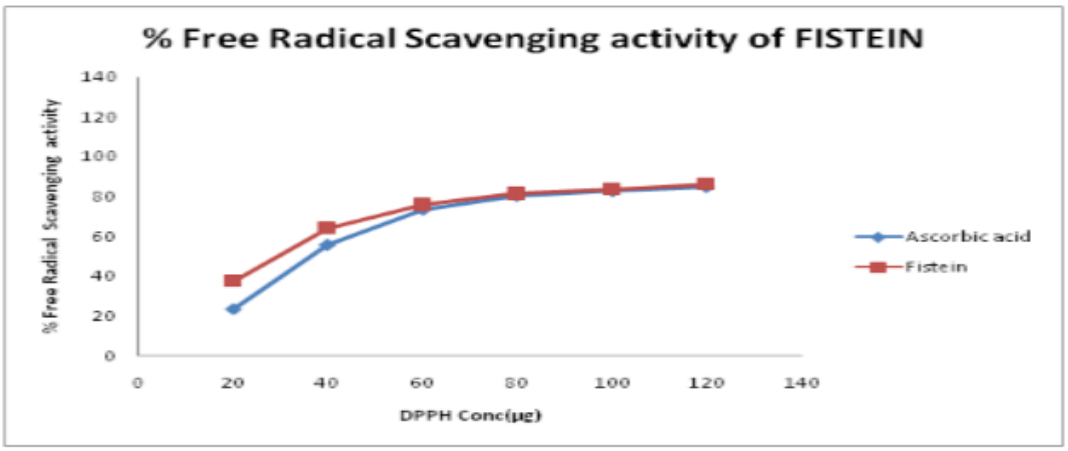


Figure No. 2: Showing % free radical Scavenging activity of different concentrations of Ascorbic acid and Fistein.

DISCUSSION

The Fistein which is present in all fruits and vegetables particularly abundant in strawberries and apples, fistein on the other hand is a naturally occurring flavonoid that has been more extensively studied in cancer; an average daily fistein intake in the Japanese diet has been estimated around 0.4 mg/day (15). In our study fisetin possesses immunosuppressant activity which is reduced the WBC Cells , the results were supporting for the previous report of Takano-Ishikawa et al. 2003., and also other reports such as the several flavonoids, namely fisetin, luteolin and apigenin (subclass of flavones), kaempherol and quercetin (flavonols), eriodictyol (flavanones), genistein (isoflavones) and butein (chalcones) exhibited inhibitory effects. Considerations on the structure of flavonoids, the C2-C3 double bond of C-ring and

4-keto functional group, were reported as essential for their inhibition of immune activities (16). Huang et al 2010 reported, flavonoids can also having the immunosuppressant property which is proved by one of the flavonoid compound Quercetin has an immunosuppressive effect on dendritic cells function (17). Flow cytometry has been changed dramatically the technical approach by which white blood cell activation could be evaluated and however very few works were done by using flowcytometry to study the effect of flavonoids on white blood cell.

Flavonoids can provide both short-term and long-term protection against oxidative stress through a variety of mechanisms. Many flavonoids act directly as antioxidants, neutralizing toxic ROS by donating hydrogen ions (18). In our study results had shown that fistein having *in vitro* antioxidant activity equal to that of Ascarbic acid, is a flavonoid compound with high antioxidant (19). Flavonoids can also having the lipid lowering property and have been found to exert several biological activities including antioxidant, anti-bacterial and anti-viral activities, and to have anti-inflammatory, anti-angionic, analgestic, hepatoprotective, cytostatic, apoptotic, estrogenic or anti-estrogenic and immune-modulating effects as well as anti-allergic properties (20-21).

CONCLUSION:

In conclusion, the flavonoid fistein posses antioxidant activity by inhibiting the generation of ROS, controlling the elevated LDL-Cholesterols suggesting a modulating the immunity against homocystein in rats.

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