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EVALUATION OF ANTI-DIABETIC AND ANTI-HYPERLIPIDEMIC ACTIVITY OF ETHANOLIC BARK EXTRACTS OF VIBURNUM OPULUS.

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ABSTRACT
Background: The present study was designed to investigate the anti-hyperlipidemic activity of Viburnum opulus extract in Triton X-100 induced hyperlipidemic rats. Phytochemical Investigation revealed the presence of Alkaloid, Tannin, Saponin, Phenol in Ethanolic Extract of Viburnum opulus while only Phenol were present in Phenolic Extract of Viburnum opulus. % Yield value of Ethanolic Extract from Aerial Parts of Viburnum opulus was found to be 17%. % Administration of Triton-X 100 (100mg/kg) to all the fasted rats caused an elevation of TC, TG, VLDL and LDL and reduction in HDL levels. After 72 hours of induction of Triton X- 100 results in hyperlipidemia which is compared with normal control group, which results in significantly increased serum lipid levels in hyperlipidemic group. The change in lipid levels in group number III to VI, were comparable with group of Hyperlipidemic control (i.e Triton X-100, Group- II). The Standard group (i.e Atorvostatin group) significantly lowers the serum lipid level ($P < 0.001$).The results of the study clearly indicate that EEVO Extract at a dose of 500 mg/kg & 400 mg/kg significantly lowered serum lipid levels ($P < 0.01$). EEVO Extract at a dose of 500 mg/kg significantly lowered serum lipid levels, ($P < 0.001$) i.e. antihyperlipidemic activity which was found to be more effective in higher dose in triton induced hyperlipidemic models. EEVO Extracts showed a dose dependent decrease in the levels of cholesterol, Triglyceride, LDL-C and VLDL-C level. Among three groups (i.e. group number III-V), Group number-V reduced the elevated lipid levels more significantly than the other groups. ($P < 0.001$) Flavonoids have exhibited a variety of pharmacological activities, including the anti-atherogenesis and antioxidant effect [48]. Thus the present result strongly suggests that the hypolipidemic activity of this medicinal plant could be attributed to the presence of Tannins, Phenols, and Flavonoids in the extracts. Methods: **Results:** The results concluded that EEVO (500 mg/kg) has a definite anti-hyperlipidemic activity in Triton X-100 induced hyperlipidemic model and which is equipotent activity when compared with Atorvastatin treated groups. Further studies on this extract may lead to identification of the possible mechanism of action and isolation of active principle from the same. Viburnum opulus has different medicinal properties and may be able to treat diabetes and diabetic complications.When subjected to acute oral toxicity studies, it was found that Viburnum opulus is safe for use up to the dose of 1000 mg/kg. It was found to act in a dose-dependent way against Alloxan induced diabetes in rats.Reduction in the elevated blood glucose levels in diabetic rats on treatment with the extract at two different concentrations confirms that Ethanolic extract of Viburnum opulus possess anti-diabetic activity and showed an equal effect when compared to Alloxan administrated rats.

Keywords: Anti-Diabetic, Anti-Hyperlipidemic, Antioxidant, Viburnum Opulus.

INTRODUCTION

Obesity has turned up as one of the major health concerns in the 21st century and is one of the leading causes of preventable death. Obesity is a term applied to excess body weight with an abnormally high proportion of body fat. Thermodynamically speaking, imbalance between energy intake (feeding) and energy expenditure (physical activity) leads to obesity. Development of obesity is, however, more complicated than that; sedentary life style, genetic factors, medical illness, microbiological aspects, social factors and neurobiological mechanisms are also involved.

Obesity has reached epidemic proportions globally, with more than 1 billion adults are overweight-at least 300 million of them clinically obese - and is a major contributor to the global burden of chronic disease and disability. Often coexisting in developing countries with under-nutrition, obesity is a complex condition, with

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serious social and psychological dimensions, affecting virtually all ages and socioeconomic groups (WHO, 2009). A growing public health concern is that the prevalence of obesity among children aged 6–19 is up to 16.5% in the USA and has also increased in Europe, Asia, Africa and South American countries. Despite increased attention given to overweight and obesity by virtually every major body concerned with public health, including the National Institutes of Health (NIH) (National Task Force, 1994) the Centers for Disease Control, the United States Department of Agriculture and the World Health Organization (World Health Organization, 2000) primary and secondary prevention efforts have generally been disappointing. Obesity impacts many facets of society. For example, it is economically costly to society (World Health Organization, 1998) increases mortality rate reduces quality of life and increases the risk of various morbidities. Extreme obesity has been estimated to truncate the lifespan of young adults by 5–20 years. The medical problems caused by obesity begin at the head and end at the toes and involve almost every organ in between. Several of these problems contribute to the earlier mortality associated with obesity and include coronary artery disease, severe hypertension that may be refractory to medical management, impaired cardiac function, adult-onset (type 2) diabetes mellitus, obesity hypoventilation and sleep apnea syndromes, cirrhosis, venous stasis and hypercoagulability with an increased risk of pulmonary embolism, and necrotizing panniculitis.[1]

Lipid is the scientific term for fats in the blood. At Normal levels, lipids perform important functions in your body, but can cause health problems if they are present in excess. The term Hyperlipidemia means high lipid levels. Hyperlipidemia includes several conditions, but it usually means that you have high cholesterol and high triglyceride levels. [1]

Etiology of obesity

The exact etiology of obesity is unclear. The multiple causative factors like genetic, environmental, nutritional, physiological, psychological, social and cultural factors have been linked to its development and progression.

Though the molecular pathways regulating energy balance are beginning to be illuminated, the causes of obesity remain elusive. In part, this reflects the fact that obesity is a heterogeneous group of disorders. At one level, the pathophysiology of obesity seems simple: a chronic excess of nutrient intake relative to the level of energy expenditure. However, due to the complexity of the neuroendocrine and metabolic systems that regulate energy intake, storage, and expenditure, it has been difficult to quantitate all the relevant parameters (e.g., food intake and energy expenditure). [2]

Environmental factors

The current environmental risk factors include over consumption of energy (increase in fat to carbohydrate ratio) and decrease in physical activity. These factors offer more reasonable explanation for the recent dramatic surge in the prevalence of obesity.

Nutritional factors

Numerous metabolic studies have shown that high fat diets may lead to a high energy intake and hyperphagia. The reason may be that fat has a weaker effect on the satiety centre and on heat production (diet-induced thermogenesis) and it possesses a higher energy density compared to carbohydrates. Also fats are highly palatable and heighten the flavour of food stuffs which leads to their passive overconsumption. This ultimately increases fat deposits and causes obesity and related problems.

Physiological factors

These involve the impairment of the central mechanism regulating appetite and food intake which is thought to be regulated by a complex interplay of neurotransmitters in the hypothalamic region of the brain. Approximately 1 - 2% of obesity can be ascribed to lesions in hypothalamic regulatory centres. Such lesions may be due to trauma, tumours, inflammatory processes, or carotid artery aneurysms.

Psychological factors

The psychogenic theory of obesity long held that obesity resulted from an emotional disorder in which food intake, relieved the anxiety and depression to which obese persons are usually susceptible. Stress associated with traumatic emotional events has been held responsible for certain cases of obesity and has been implicated in the pathogenesis of eating disorders such as night-eating syndrome and bulimia. [3]

Role of Genes versus Environment

Obesity is commonly seen in families, and the hereditability of bodyweight is similar to that for height. Inheritance is usually not Mendelian, however, and it is difficult to distinguish the role of genes and environmental factors. Adoptees usually resemble their biologic rather than adoptive parents with respect to obesity, providing strong support for genetic influences. Likewise, identical twins have very similar BMIs (Body Mass Index) whether reared together or apart, and their BMIs are much more strongly correlated than those of dizygotic twins. These genetic effects appear to relate to both energy intake and expenditure.

Whatever the role of genes, it is clear that the environment plays a key role in obesity, as evidenced by the fact that famine prevents obesity in even the most obesity-prone individual. In addition, the recent increase in the prevalence of obesity in the United States is too rapid to be due to changes in the gene pool. Undoubtedly, genes influence the susceptibility to obesity when confronted with specific diets and availability of nutrition. Cultural factors are also important these relate to both variability and composition of the diet and to changes in the level of physical activity. In industrial societies, obesity is more common among poor women, whereas in underdeveloped countries, wealthier women are more often obese. In children, obesity correlates to some degree with time spent

watching television. High fat diets may promote obesity, as many diets rich in simple (as opposed to complex) carbohydrates. [4]

Specific Genetic Syndromes

For many years obesity in rodents has been known to be caused by a number of distinct mutations distributed through the genome. Most of these single-gene mutations cause both hyperphagia and diminished energy expenditure, suggesting a link between these two parameters of energy homeostasis. Identification of the ob gene mutation in genetically obese (ob/ob) rats represented a major breakthrough in the field. The ob/ob mouse develops severe obesity, insulin resistance, and hyperphagia, as well as efficient metabolism (e.g., it gets fat even when given the same number of calories as lean littermates). The product of the ob gene is the peptide leptin, a name derived from the Greek root leptos, meaning thin. Leptin is secreted by adipose cells and acts primarily through the hypothalamus. Its level of production providesan index of adipose energy stores. High leptin levels decrease food intake and increase energy expenditure. Another mouse mutant, db/db, which is resistant to leptin, has a mutation in the leptin receptor and develops a similar syndrome.

The OB gene is present in humans and is expressed in fat. Several families with morbid, early-onset obesity caused by inactivating mutations in either leptin or the leptin receptor have been described, thus demonstrating the biologic relevance of leptin in humans. The obesity in these individuals begins shortly after birth, is severe, and is accompanied by neuroendocrine abnormalities. The most prominent of these is hypogonadotropic hypogonadism, which is reversed by leptin replacement. Central hypothyroidism and growth retardation are seen in the mouse model, but their occurrence in leptin-deficient humans is less clear. To date, there is no evidence to suggest that mutations or polymorphisms in the leptin or leptin receptor genes play a prominent role in common forms of obesity. Mutations in several other genes cause severe obesity in humans; each of these syndromes is rare. Mutations in the gene encoding proopiomelanocortin (POMC) cause severe obesity through failure to synthesize a-MSH, a key neuropeptide that inhibits appetite in the hypothalamus. The absence of POMC also causes secondary adrenal insufficiency due to absence of adrenocorticotropic hormone (ACTH), as well as pale skin and red hair due to absence of MSH. Proenzyme convertase 1 (PC-1) mutations are thought to cause obesity by preventing synthesis of a-MSH from its precursor peptide, POMC. a- MSH binds to the type 4melanocortin receptor (MC4R), a key hypothalamic receptor that inhibits eating. Heterozygous mutations of this receptor appear to account for as much as 5% of severe obesity. These five genetic defects define a pathway through which leptin (by stimulating POMC and increasing MSH) restricts food intake and limits weight.

In addition to these human obesity genes, studies in rodents reveal several other molecular candidates for hypothalamic mediators of human obesity or leanness. The tub gene encodes ahypothalamic peptide of unknown function; mutation of this gene causes late-onset obesity. The fat gene encodes carboxypeptidase E, a peptide-processing enzyme; mutation of this gene is thought to cause obesity by disrupting production of one or more neuropeptides. AgRP is co-expressed with NPY in arcuate nucleus neurons. AgRP antagonizes a- MSH action at MC4 receptors, and its overexpression induces obesity. In contrast, a mouse deficient in the peptide MCH, whose administration causes feeding, is lean. A number of complex human syndromes with defined inheritance are associated with obesity. Although specific genes are undefined at present, their identification will likely enhance our understanding of more common forms of human obesity. In Prader-Willi syndrome, obesity coexists with short stature, mental retardation, hypogonadotropic hypogonadism, hypotonia, small hands and feet, fish-shaped mouth, and hyperphagia. Most patients have a chromosome 15 deletion. Laurence-Moon-Biedl syndrome is characterized by obesity, mental retardation, retinitis pigmentosa, polydactyly, and hypogonadotropic hypogonadism. [4]

Diabetes is a metabolic disorder which can be considered as a major cause of high economic loss which can in turn impede the development of nations. Moreover, uncontrolled diabetes leads to many chronic complications such as blindness, heart failure, and renal failure. In order to prevent this alarming health problem, the development of research into new hypoglycemic and potentially anti diabetic agents is of great interest.

In the last few years there has been an exponential growth in the field of herbal medicine and these drugs are gaining popularity both in developing and developed countries because of their natural origin and less side effects. Many traditional medicines in use are derived from medicinal plants, minerals and organic matter. A number of medicinal plants, traditionally used for over 1000 years named “rasayana” are present in herbal preparations of Indian traditional health care systems. In Indian systems of medicine most practitioners formulate and dispense their own recipes. The World Health Organization (WHO) has listed 21,000 plants, which are used for medicinal purposes around the world. Among these 2500 species are in India, out of which 150 species are used commercially on a fairly large scale. India is the largest producer of medicinal herbs and is called as botanical garden of the world. The current review focuses on herbal drug preparations and plants used in the treatment of diabetes mellitus, a major crippling disease in the world leading to huge economic losses.

Aim and Objectives

Evaluation of *Viburnum opulus* extracts on plasma lipid levels and glucose levels in Triton -X 100 induced hyperlipidemic rats.

The evaluation of *Viburnum opulus* for Anti-diabetic and Anti-hyperlipidemic activity in rats involves:

- Acquiring and drying of Plant Material (*Viburnum opulus)*
- Extraction & Phytochemical Screening of *Viburnum opulus.*

- Toxicity Study of *Viburnum opulus* extract on Wister rats
- To induce hyperlipidemia by Triton X-100 in Wister rats.
- To evaluate Anti-hyperlipidemic activity of *Viburnum opulus* extracts in Triton x-100 induced hyperlipidemic rats.
- To determine the *Viburnum opulus* diabetic properties.

Method
Materials

- AtorvastatinDr.Reddys Lab,Hyderabad.
- Metformin MSN Formulations,HYD,India
- Gold thio glucose Sigma, St Louis, U.S.A.
- Normal saline Claris life sciences .Ltd., Ahmedabad, India.
- Chloroform Molychem, Mumbai.
- Diethyl ether Finar Ltd, Ahmedabad, India.
- Triton X-100 Unisource Chemical Pvt, Ltd
- Shimadzu Electronic Balance Toshvin Analytical Pvt.Ltd, Mumbai, India
- U.V spectrophotometer Shimadzu UV-1800.
- Ultra-homogenizer Biologics,inc, USA.
- Centrifuge RM-12C Remi Electro Technik Ltd, Mumbai India.
- Rotary Evaporator Heidolph Rotary Flask Evaporator,India.
- Total cholesterol kits Excel Diagnostics Pvt, Ltd, India
- Triglycerides Kits Excel Diagnostics Pvt, Ltd, India
- HDL-Cholesterol Kits Excel Diagnostics Pvt Ltd , India
- Plant materials
- Domain: Eukaryota
- Kingdom: Plantae
- Phylum: Tracheophyta
- Subphylum: Angiospermae
- Class: Magnoliopsida
- Kingdom: Plantae
- Family: Adoxaceae
- Genus: Viburnum

1. Description:

There are three cytotypic forms distinguished by chromosome number: a diploid form (2n=24), an infertile triploid form (2n=36), and a tetraploid form. The triploid form is the most common and is thought to have arisen relatively recently in the Himalayan region through hybridisation of the diploid with the tetraploid.

Viburnum opulus has been an item of trade in many cultures for thousands of years. It has been used medicinally for a wide variety of ailments, and its aroma makes guelder rose essential oil valued in the perfume industry. In the 19th century, cramp bark was adopted for treatment of stomach cramps and dysmenorrhea, and to prevent miscarriage. Fermented fruit juice of European cranberry bush is useful for kidney disorders, menstrual and stomach cramps, hypertension, asthma, digestion problems, and the common cold. In Europe Viburnum opulus was often added to wine, and the root is also one of the possible ingredients of absinthe. It is also used in bitters.[4]

The bright red berries are deeply rooted in natural mysticism and folklore of Europe and Asia, and the bark, roots, berries, and leaves are used medicinally, in culinary applications, and in religious practices. The leaves, stems, and roots are used in various Siddha and Ayurvedic medicines. It is widely employed in modern herbal medicine for sedative, laxative, diuretic, and carminative properties. It is used in Ayurveda to counter the side effects of all hallucinogens.[36] The bark contains ‘scopoletin’, a coumarin that has a sedative effect on the uterus.

Viburnum opulus shows neuroprotective effect against stroke and chemically induced neurodegeneration in rats. Specifically, it has protective effect against acrylamide-induced neurotoxicity.

Both roots & leaves of Viburnum opulus have shown antioxidant, antimicrobial and insecticidal activities.

Viburnum opulus may prove to be an effective control measure against cattle tick, Rhipicephalus (Boophilus) microplus.

A recent study showed that beta-asarone isolated from Viburnum opulus oil inhibits adipogenesis in 3T3-L1 cells and thus reduces lipid accumulation in fat cells.

2. Constituents:

Our previous studies identified Viburnum opulus fruit as a rich source of phenolic compounds, with chlorogenic acid, proanthocyanidins and catechins as the main constituents, as shown in Figure. Despite their high antioxidant potential, Viburnum opulus phenolics were able to decrease intracellular oxidative stress and inhibit cell migration [24–s such as alkaloids, flavonoids, gums, lectins, mucilage, phenols, quinone, saponins, sugars, tannins, and triterpenes (steroids).Active constituents include flavonoids, lectins, phenols, and saponins. There was detection of flavonoids and phenols as inhibitors of the growth of methicillin- resistant Staphylococcus aureus; in an alcoholic extract with this antibacterial action, flavonoids and phenols were the major active constituents. Lectins had mitogenic action on mononuclear cells from the peripheral blood of healthy humans and on murine splenocytes (macrophages of murine spleen). With lectins, there also was inhibition of the growth of some neoplastic cell lines from rats. Saponins produced effects against hyperlipdemia in rats.

MATERIALS AND METHOD

1. Collection and Authentification of Plant Material

The Aerial Parts of Viburnum opulus were collected and authenticated.

2. Preparation of Extract

After cleansing the whole plant of Viburnum Opulus was cut down into small pieces and dried under shade for 3 weeks. After drying the plant was ground into coarse powder by using grinding machine (National,

Model MJ-176NR, China) and 500 g powdered material was soaked into 3 L of 99.9% ethanol for maceration purpose at room temperature. After three days the soaked material was filtered first with muslin cloth and then re-filtered with Watmann Grade-1 filter paper. Residue was dried to evaporate ethanol. After three days filtrate was obtained. Ethanolic filtrates were subjected rotary evaprator. At the end semisolid paste like extracts (blackish green ethanolic extract and golden brown aqueous extract) were obtained. The extract was kept in vacuum desiccator for 7 days for removal of moisture, which were then preserved in refrigerator after sealing in air tight containers with proper labeling. [14] The purpose of using ethanol as solvent was to extract maximum of phytochemical constituents from plant. % Yield for ethanolic extract was found to be 17%. It was calculated as: Percentage (%) Yield = Weight of extract (g) / Weight of powder (g) X 100

3. Preliminary Phytochemical Screening

Preliminary phytochemical screening of the Viburnum opulus extract was carried out for the analysis of Alkaloids, Carbohydrates, Tannins, Saponins, Steroids, Phenols, and Flavonoids as per the standard methods. [40]

4. Animals

Healthy Adult Male Wistar rats of 8-10 weeks old with Average weight in the range of 150- 180gms were selected. Animals are housed 4 per cage in temperature controlled (270C ± 30C) room with light/dark cycle in a ratio of 12:12 hours is to be maintained. The Animals are allowed to acclimatize to the environment for seven days and are supplied with a standard diet and water ad libitum. The prior permission was sought from the Institutional Animal Ethics Committee (IAEC) for conducting the study.

5. Acute toxicity studies

The Acute oral toxicity test of the extracts was determined prior to the experimentation on animals according to the OECD (Organization for Economic Co-operation and Development) guidelines no 423. Female Albino Wistar rats (130-200 g) were taken for the study and dosed once with 2000 mg/kg of the extract. The treated animals were monitored for 14 days to observe general clinical signs and symptoms as well as mortality. No mortality was observed till the end of the study revealing the 2000 mg/kg dose to be safe. Thus, ¼ and 1/8 doses of 2000 mg/kg i.e. 500 mg/kg and 250 mg/kg were chosen for subsequent experimentation.

6. Method of Induction

The systemic administration of the surfactant Triton X-100 to rats and supportive high fat diet results in elevation of plasma cholesterol and triglycerides. Hyperlipidemia was induced in Wistar albino rats by single i.p injection of freshly prepared solution of Triton-X-100 (100 mg/kg) in physiological saline solution after overnight fasting for 18 hours. And it is supplied with high fat diet. [13]

7. Experimental Animal Protocol

Experimental rats, starved for 18 hours, were provided water ad libitum. The rats were divided into five groups containing four animals in each group.

- Group-I: Normal Control (Normal saline 10ml/kg orally) for 7 days.
- Group-II: Hyperlipidemic control, three i.v. injection of Triton-X-100 on consecutive days with high fat diet.
- Group-III: Hyperlipidemic Rats treated with Atorvastatin (Standard drug) at 10 mg /kg orally for 7 days with high fat diet.
- Group-IV: Hyperlipidemic rats treated with Ethanolic extract of Viburnum opulus (Low Dose) at a daily dose of 250 mg/kg orally for 7 days with high fat diet.
- Group-V: Hyperlipidemic Rats treated with Ethanolic extract of Viburnum opulus (High Dose) at a daily dose of 500 mg/kg orally for 7 days with high fat diet.

The rats were divided into five groups containing four animals in each group. All the groups receives three i.p injection of Triton-X 100 (100 mg/kg) on consecutive days with supportive high fat diet , simultaneously with Group- II, Group – III, Group – IV, Group – V, expect Group – I (Normal control) to induce hyperlipidemia.

The Group – III receives atorvastatin at dose of 10 mg/kg, was prepared by suspending bulk atorvastatin in aqueous 5% methyl cellulose for 7 days. The Group– IV, receive Viburnum opulus at a dose of 250 mg/kg for 7 days and Group – V, receives Viburnum opulus at a dose of 500mg/kg for 7 days.

8. Experimental procedure of diabetes induction

After 1 week of acclimatization, rats of each strain were divided into control or GTG groups. As the expected rates of GTG-induced obesity ranged from 40 to 80% depending on the mouse strain, a large number of rats (12–20 rats) were assigned to GTG groups with the aim of obtaining six to eight obese rats per group. The rats received an intraperitoneal administration of saline or GTG (Cat. No. 1045508; USP, Rockville, MD) at the following optimal doses: 0.6, 0.8, 0.4, and 0.8 g/kg for rats groups, respectively. After 4 or 6 weeks, rats that developed obesity (GTG-obese rats) were selected for further studies on showing an 8 g or greater weight gain compared with the average weight gain measured in the control rats. The development of obesity-induced diabetes was investigated in control and GTG-obese rats of each strain (n = 6–8 per group) for 12–14 weeks. Body weights were measured every 2 or 4 weeks and the development of diabetes was monitored by measuring non fasting plasma glucose levels between 9:00 and 10:30 am.

9. Experimental Study Design for Diabetic screening

Diabetic rats were divided in to five groups with each group four animals:

Group-I: Rats served as normal control group.
Group-II: Served as diabetic/disease control.

Group-III: Diabetic rats treated with Viburnum opulus at a dose 250 mg/kg. Group-IV: Diabetic rats treated with Viburnum opulus at a dose of 500 mg/kg Group V: Diabetic rats treated with Metformin (standard drug) at 400 mg/kg.

The treatment was given for 14 days and blood samples were collected at different intervals.

10. Collection of blood samples

Blood samples were collected from all the groups of animals at 0, 7,15th day intervals through puncturing of retro orbital plexus and were centrifuged at 3000 revolutions per minute (rpm) for 15 minutes. Serum was separated and stored at -20^{0}c and then used for estimating blood glucose levels.

Evaluation parameter

- GLUCOSE[23]Method: GOD/POD method

Principle:

- D-glucose + H2O + O2 $\xrightarrow{\text{Glucose oxidase (GOD)}}$ Gluconic acid + H2O2
- H2O2 + 4-AAP + Phenol $\xrightarrow{\text{Peroxidase (POD)}}$ Quinoneimine dye + H2O

Procedure:

- Wavelength/filter: 505 nm (Hg 546 nm) / Green
- Temperature: 37^0 C / R.T.
- Light path: 1 cm
- Pipette into clean dry test tubes labeled as Blank (B), Standard (S) and Test (T)

Table.no:2: Addition sequence and required quantities for GOD/POD method:

Mix well and incubate at 37^{0}C for 10 min or at R.T. (25^{0}C) for 30 mins. Measure absorbances of the Standard (Abs.S) and Test Sample (Abs.T) compare these against the Blank within 60 minutes.
Blood Sample Collection and Analysis

The rats are anesthetized by ether and then Blood samples were collected on 0th and 8th day [13] from retro-orbital plexus of rat using micro capillary technique from rats of all the groups [43], and centrifuged at 3000 rpm for 15 min so as to get serum. The serum is analyzed for total cholesterol, triglycerides and HDL levels using biochemical kits (diagnostic kit). [44]VLDL, and LDL –

Cholesterol were calculated by the below formula:

Serum LDL - Cholesterol concentration was calculated According to the equation of Fried and wald45.
LDL – Cholesterol = Total Cholesterol – (HDL-Cholesterol +TG/5) VLDL-C = TG/5

2. Bio Chemical Assays for lipids

Estimation Procedures: Plasma Lipid Profile Estimation

Total cholesterol LDL cholesterol, HDL Cholesterol, VLDL cholesterol, Triglycerides levels were measured using commercial kits.

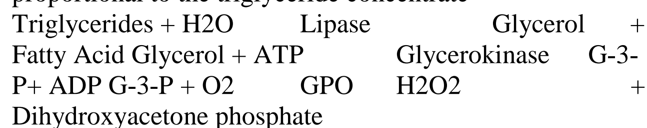
Estimation of Triglycerides. (GPO/PAP Method) Clinical Significance

Determination of serum Triglycerides concentration is used to assess the possible presence of increased blood and Serum levels of triglycerides.

Principle

Triglycerides are hydrolysed by lipase to glycerol and free fatty Acids.

Glycerol is phosphorylated by ATP in the presence of glycerolkinase (GK) to glycerol – 3 – phosphate (G-3-P) which is oxidized by the enzyme Glycerol–3–phosphoxidase (G-P-O) producing hydrogen peroxide. Hydrogen peroxide so formed reacts with 4-Amino-Hydrogen peroxidave (POP), to produce a brown colour complex. The intensity of the colour developed is proportional to the triglyceride concentrate



Triglycerides + H2O Lipase Glycerol + Fatty Acid Glycerol + ATP Glycerokinase G-3-P+ ADP G-3-P + O2 GPO H2O2 + Dihydroxyacetone phosphate

H2O2 + ESPAS + 4 – AAP POD Brown Colour + H2O.

Procedure

Wave length: 546 (Green Filter)Temperature: 37 C Reaction type: End point with standard.
Pipette in to clean drytube labelled Blank (B), Standard (S) and Test (T) and then add following:

Table.no:3: Addition sequence and required quantities for plasma lipid profile estimation:

Mix well and incubate for 10 minute at 370 C. Read absorbance of standard and test against blank.
Calculations

Triglyceride concentration in mg % = Absorbance of test /Absorbance of Standard X 200

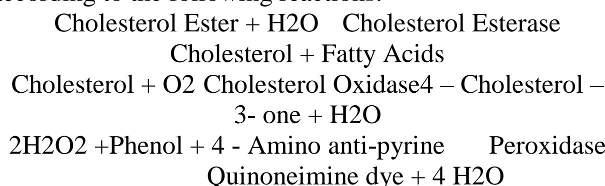
3. Estimation of cholesterol (Total cholesterol). CHOD/POD Method:

Clinical Significance

Heart disease is often the result of cholesterol deposits on the arteries. While not the only factor for heart disease, serum cholesterol levels are often checked to determine the risk of heart disease on patient.

Principle

Enzymatic determinations of total cholesterol according to the following reactions:-



Cholesterol Ester + H2O Cholesterol Esterase Cholesterol + Fatty Acids
Cholesterol + O2 Cholesterol Oxidase4 – Cholesterol – 3- one + H2O
2H2O2 +Phenol + 4 - Amino anti-pyrine Peroxidase Quinoneimine dye + 4 H2O

Procedure

Wave Length: 500 nm (green filter) Temperature: 370C. Reaction type: End point with standard.

Table.no:4: Addition sequence and required quantities for CHOD/POD method:

Pipette in a clean dry test tube labelled as Blank (B), Standard (S), and Test (T).

Mix and read the optical density (OD) at 500 nm against blank after 5min incubation (370C). The final colour is stable for at least 1 hour.

Calculations

Cholesterol concentration in mg% = Absorbances of Test/Absorbances of Standard×200 (Standard).

4. Estimation of HDL cholesterol [47] Procedure: It includes two steps. Step: 1- Precipitation
Step: 2 – Colour development
Take 3 clean glass tubes labelled as blank (B), standard (S), and test (T).
Mix well and stand at room temperature for 10 min, centrifuge at 3000 rpm for 10 min.
Incubation for 5 minutes at 370C and read the optical density at 500 nm against blank.

Calculations

HDL cholesterol =Absorbance of test /Absorbance of standard × 50 (Standard concentration).

5. LDL CALCULATION [47]

It is calculated using formula: LDL = TC-HDL-TG/5.0 (mg/dl). VLDL is calculated using formula:
VLDL = Triglycerides (mg/dl) / 5,

According to these guidelines, the normal range of lipid profile:
LDL/HDL and TC/HDL ≤ 5 mg/dl are the favourable risk factor.

6. Statistical Analysis

Results are expressed as Mean ± S.D .all the results were compared with control subject one- way analysis of variance (ANOVA), followed by the Dunnet t-test using Graph Pad Prism Software 6 version. P Values < 0.05 were as considered statistically significant.

7. RESULTS AND DISCUSSION

% Yield of Ethanolic Extract from Aerial Parts of *Viburnum opulus* was found to be 17%
Preliminary Phytochemical Screening
Investigation revealed the presence of Alkaloid, Tannin, Saponin, and Phenol in Ethanolic extract of *Viburnum opulus.*

8. Acute toxicity studies

As per (OECD) draft guidelines 423 adopted, Female albino rats were administered with *Viburnum opulus* and doses was be selected in the sequence (1.75-5000) using the default dose progression factor, for the purpose of toxicity study. Animals are observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours and daily thereafter, for a total of 14 days,. In all the cases, no death was observed within 14 days. Additional observations like behavioral changes in skin, fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous systems and somatic motor activity and behavior pattern were also found to be normal. Attention was also given to observation of tremors and convulsions, salvation, diarrhea, lethargy, sleep and coma. Overall results suggested the LD50 value to be 5000 mg/kg. Hence therapeutic dose was calculated (i.e. 400 g/kg and 500 mg/kg) of the lethal dose for the purpose of anti-hyperlipidemic investigations.

Fig 1: NHLBI's classification of BMI

NHLBI Terminology[11]	BMI, kg/m^2, Range	WHO Classification[1]
Underweight	<18.5	Underweight
Normal	18.5-24.9	Normal range
Overweight	25.0-29.9	Preobese
Obesity class 1	30.0-34.9	Obese class 1
Obesity class 2	35.0-39.9	Obese class 2
Obesity class 3	≥40.0	Obese class 3

*NHLBI indicates National Heart, Lung, and Blood Institute and WHO, World Health Organization.

Table.no:2: Addition sequence and required quantities for GOD/POD method:

Addition Sequence	**B (ml)**	**S (ml)**	**T (ml)**
Glucose Reagent (L1)	1.0	1.0	1.0
Distilled Water	0.01	--	--
Glucose Standard (S)	--	0.01	--
Sample	--	--	0.01

Table.no:3: Addition sequence and required quantities for plasma lipid profile estimation:

	Blank	Standard	Test
Enzyme reagent	1.0 ml	1.0 ml	1.0 ml
Standard	-	0.01ml	-
Serum / Plasma	-	-	0.01 ml

Table.no:4: Addition sequence and required quantities for CHOD/POD method:

	Blank	Standard	Test
Enzyme Reagent	1ml	1ml	1ml
Deionized water	0.01ml	-	-
Standard	-	0.01ml	-
Serum/plasma	-	-	0.01ml

Table.no:5: Precipitation test for HDL cholesterol:

Serum	0.2 ml
HDL precipitating reagent	0.3 ml

Table.no:6: Colour development test for HDL cholesterol:

	Blank	Test	Standard
Enzyme reagent	1 ml	1 ml	1 ml
Cholesterol (Standard)	-	0.01 ml	-
Supernatant serum Step -1	-	-	0.1 ml
Distilled water	0.1 ml	0.1 ml	-

Table.no:7: Guidelines for normal range of lipid profile:

Total cholesterol	< 200 mg/dl
Triglycerides	< 200 mg/dl
HDL	> 40 mg/dl
LDL	< 150 mg/dl
VLDL	5-30 mg/dl

Table.no:8: Preliminary Phytochemical Screening:

Phytochemical screening	Results
Steroid	-
Alkaloid	+
Tannin	+
Carbohydrate	-
Phenol	+
Flavonoid	+
Saponin	+

Table.no:9: Lipids Levels Obtained on 8th Day (After Treatment):

S.No	Groups	TC	TG	HDL	LDL	VLDL
I	Normal Control	64.03 ±1.45	82.66±2.46	38.91±2.33	8.45±3.34	16.53±0.49
II	Hyperlipidemic Control	192.47±5.05	168.9±5.28	21.86±2.74	136.82±7.00	33.79±1.05
III	EEVO500 mg/kg	134.19±3.5*	117.57±5.25*	27.1±2.99***	83.58±5.26*	23.51±1.05***
IV	EEVO 250 mg/kg	121.74±7.74 *	107.93±6.67*	31.04±4.32***	69.11±10.51***	21.58±1.33***
V	Atorvastatin	134.69±4.0*	118.07±6.05*	27.6±3.49***	84.08±5.76*	24.01±1.55***

Table.no:10: Effect of Viburnum opulus (EEVO) on serum glucose levels (mg/dl) in diabetic rats:

Groups/Interval	0th Day	7th Day	15th Day
Normal	83.3 ± 4.23	79.1 ± 5.36	77.7 ± 5.62
Diabetic control	283.8 ± 5.01	286.4 ± 12.4	300.3 ± 8.64
EEVO (500 mg/kg)	293.1 ± 9.83	192.1 ± 12.3**	100.3 ± 12.5**
EEVO (250 mg/kg)	280.5 ± 42.4	185.2 ± 11.2***	94.2 ± 7.2***
Metformin (400 mg/kg)	271.0 ± 13.5	80.2 ± 6.4***	70.1 ± 6.3**

Results:
All the data are expressed as MEAN ± S.D (n=4),*P = 0.001, **P = < 0.01, ***P = < 0.05 vs GROUP II

TC: Total Cholesterol; TG: Triglycerides; HDL-C: High Density Lipoprotein cholesterol;
LDL-C: Low Density Lipoprotein- cholesterol; VLDL-C: Very Low Density Lipoprotein;
EEVO: Ethanolic Extract of Viburnum opulus.

2. Serum glucose levels

All the values of mean ± SD; n=6; ** indicates p<0.01, *** indicates ap <0.001 vs diabetic control.

2. Discussion

The present study was designed to investigate the antihyperlipidemic activity of Viburnum opulusextract in Triton X-100 induced hyperlipidemic rats. Phytochemical Investigation revealed the presence of Alkaloid, Tannin, Saponin, Phenol in Ethanolic Extract of Viburnum opuluswhile only Phenol were present in Phenolic Extract of Viburnum opulus %Yield value of Ethanolic Extract from Aerial Parts of Viburnum opulus was found to be 9.27%. % Administration of Triton-X-100 (100mg/kg) to all the fasted rats caused an elevation of TC, TG, VLDL and LDL and reduction in HDL levels. After 72 hours of induction of Triton X- 100 results in hyperlipidemia which is compared with normal control group, which results in significantly increased serum lipid levels in hyperlipidemic group. The change in lipid levels in group number III to VI, were comparable with group of Hyperlipidemic control (i.e Triton X-100, Group- II). The Standard group (i.e Atorvostatin group) significantly lowers the serum lipid level (P < 0.001).

The results of the study clearly indicate that EEVO Extract at a dose of 500 mg/kg & 250 mg/kg significantly lowered serum lipid levels (P < 0.01). EEVO Extract at a dose of 500 mg/kg significantly lowered serum lipid levels, (P < 0.001) i.e. anti-hyperlipidemic activity which was found to be more effective when administered orally in triton induced hyperlipidemic models.

EEVO Extract has a very low hypolipidemic activity. EEVO Extracts showed a dose dependant decrease in the levels of cholesterol, Triglyceride, LDL-C and VLDL-C level. Among three groups (i.e. group number III-V), Group number- V reduced the elevated lipid levels more significantly than the other Groups (P < 0.001).

Flavonoids have exhibited a variety of pharmacological activities, including the anti-atherogenesis and antioxidant effect.[48] Thus the present result strongly suggests that the hypolipidemic activity of this medicinal plant could be attributed to the presence of Tannins, Phenols, and Flavonoids in the Extracts.

REFERENCES

1. Nimmy Chacko, Shastry CS, Prerana shetty, Prasanna Shyamma, Ullas D'souza and Patel Maulika, *et al.* Anti hyperlipidemic Activity of Costus Igneus in Triton X-100 Induced Hyperlipidemic Rats. *Int Jour of Pharm and Chem Scienc*. 1(2), 2012, 813-818.
2. S.S.Sudha, R.Karthic, Naveen, J.Rengaramanujam, *et al.* Anti hyperlipidemic activity of Spirulina platensis in Triton x-100 induced hyperlipidemic rats. *Hygeia.J.D.Med*. 3(2), 2011, 32-37.
3. Krishna Chaitanya B, Raavindra Babu, Jayasree Vardhana, Alekhya Ravellaa, Diana Vivian Atigaria, and Jaji Sreeb, *et al.* Antihyperlipidemic activity of Ruellia Tuberosa Linn in triton induced hyperlipidemic rats. *Int J Pharm*. 2(4), 2012, 740-745.
4. Dhanaji D. Ghanwat, Jayant S. Bidkar, Madhuri D. Bhujbal, Ganesh Y. Dama, *et al.* Anti- Hyperlipidemic Activity of Cucumis melo Fruit Peel Different Extracts in Triton X-100 Induced Hyperlipidemia in Rats. *Int Jour Uni Phar & Bio Sci.* 1(1), 2012.

5. Society for Vascular Surgery. (Home page on Internet) Reviewed December 2010. http://www.vascularweb.org/vascularhealth/Pages/hyperlipidemia.aspx/.NutritionMD http://www.nutritionmd.org/health_care_providers/cardiovascular/hyperlipidemia_diagnosis.html.
6. Sharma N and Garg V, *et al*. Antidiabetic and Anti-oxidant potential of ethonolic extract of butea monosperma leaves in alloxan induced diabetic rats. *Ind J.Biochem Biophys.* 46(1), 2009, 99-105.
7. Ram Niwas Jangir, Gyan Chand Jain, *et al.* Evaluation of Antidiabetic Activity of Hydroalcoholic Extract of Cassia fistula Linn. pod in Streptozotocin-Induced Diabetic Rats, *Pharmacognosy Journal*, 9(5), 2017, 599-606.
8. P. Jaya Preethi. Herbal medicine for diabetes mellitus: A Review, *International Journal of Phytopharmacy*. 3(1), 2013, 01-22.
9. Anil Kamboj, Sunil Kumar, and Vipin Kumar, *et al.* Evaluation of Antidiabetic Activity of Hydroalcoholic Extract of Cestrum nocturnum Leaves in Streptozotocin-Induced Diabetic Rats. *Advances in Pharmacological Sciences*, l, 2013, 4.
10. Taofik O. Sunmonu and Anthony J. Afolayan, *et al.* Evaluation of Antidiabetic Activity and Associated Toxicity of Artemisia afra Aqueous Extract in Wistar Rats. *Evidence-Based Complementary and Alternative Medicine*, l, 2013, 8.
11. Manisha Modak,1 Priyanjali Dixit,1 Jayant Londhe,1 Saroj Ghaskadbi, 1 and Thomas Paul A, *et al.* Devasagayam, Indian Herbs and Herbal Drugs Used for the Treatment of Diabetes, *J Clin Biochem Nutr*. 40(3), 2007, 163–173.
12. Society for Vascular Surgery. (Home page on Internet) Reviewed December 2010. http://www.vascularweb.org/vascularhealth/Pages/hyperlipidemia.aspx/ NutritionMD:http://www.nutritionmd.org/health_care_providers/cardiovascular/hyperlipidemia_diagnosis.htl.
13. Sharma N and Garg V, *et al.* Antidiabetic and Anti-oxidant potential of ethonolic extract of butea monosperma leaves in alloxan induced diabetic mice. *Ind J.Biochem Biophys*. 46(1), 2009, 99-105.
14. Kumar a.s, A Mazumder and V.S Saravanam, *et al.* Anti hyperlipidemic activity of camellia sinensis leaves in triton wr-1339 induced albino rats. *pheg.mag*, 4, 2008, 60-64.
15. Jain k.s, M.k. kathiravan, R.S Somani and C.J.Shishoo, *et al.* The biology and chemistry of hyperlipedemia. Bio.Med chem. 15, 2007, 4674-4699.
16. Davey smith, and Gand j pekkanen, *et al.* should there be a moratorium on the use of cholesterol lowering drugs. *Br.Med.J*, 304, 1992, 431-740.
17. H.P.Rang, M.M.Dal, J.M.Ritter, R.J.Flower, *et al.* Rang & dale’s Pharmacology; ELSEVIER Imprints, Philadelphia, 7th Edition, 2007. 285-292, 303-306.
18. News-Medical.Net.http://www.news-medical.net/health/What-is-Hyperlipidemia.aspx. Accesed on Dated 12/02/2013.
19. News-Medical.Net.http://www.news-medical.net/health/What-is- Hyperlipidemia.aspx. Accesed on Dated 12/02/2013.
20. 20. NutritionMD Accessed on Dated 10/02/2021 Available from: https://www.nutritionmd.org/health_care_providers/cardiovascular/hyperlipidemia.html. Accessed on Dated 10/02/2021.
21. Brown, S.L. Lowered serum cholesterol and low mood. *Br.Med.J*, 313, 1996, 637-638.
22. Berlinear, j.a and Y.Suzuki, *et al*. The role of oxidized lipoprotein in atherogenesis, *free radical. Bio.Med*. 20, 1996,707-727.
23. Nimmy Chacko, Shastry CS, Prerana shetty, Prasanna Shyamma, Ullas D’souza and Patel Maulika, *et al.* Anti hyperlipidemic Activity of Costus Igneus in Triton X-100 Induced Hyperlipidemic Rats. *Int Jour of Pharm and Chem Scienc.* 1(2), 2012, 813-818.
24. S.S.Sudha, R.Karthic, Naveen, J.Rengaramanujam, *et al.* Anti hyperlipidemic activity of Spirulina platensis in Triton x-100 induced hyperlipidemic rats. *Hygeia.J.D.Med*. 3(2), 2011, 32-37.
25. Krishna Chaitanya B, Raavindra Babu, Jayasree Vardhana, Alekhya Ravellaa, Diana Vivian Atigaria, and Jaji Sreeb, *et al.* Antihyperlipidemic activity of Ruellia Tuberosa Linn in triton induced hyperlipidemic rats. *Int J Pharm* 2(4), 2012, 740-745.
26. Dhanaji D. Ghanwat, Jayant S. Bidkar, Madhuri D. Bhujbal , Ganesh Y. Dama, *et al.* Anti- Hyperlipidemic Activity of Cucumis Melo Fruit Peel Different Extracts in triton x-100 induced hyperlipidemia in rats. *Int Jour Uni Phar & Bio Sci*. 1(1), 2012.
27. Riadh ksouri, Hanen falleh, Wided meg diche, Najla trabelsi, Baya mhamdi, Kamel chaieb, Amina bakrouf, Christian magne, and Chedly abdelly, *et al.* Antioxidant and antimicrobial activities of the edible medicinal halophyte tamarix gallica .L and related polyphenolic compounds. *ELSEVIER Food and Chem Toxi* .47(8), 2009, 2083-2091.
28. Butea monosperma (Lam.) Taub. Germplasm Resources Information Network. United States Department of Agriculture. 2006-05-18. Retrieved 2009-10-24.
29. Dr.Salima benhouhou. A Guide to medical plants in north Africa. 265-266. http:/www.uicnmed.org/nabp/database/htm/pdf/p64.pdf. accesed on dated 15- 03-2013.
30. Profile for Tamarix gallica [French tamarisk].plants database, usda, nrccs. http://plants.usda.gov/java/profile?symbol=TAGA;retrived accesed on dated 11-03-2013.
31. Qureshir and g.r bhatti, *et al.* Diversity of micro-habitats and their plants resources, *in nara desert pak.j.bot*, 40, 2008, 979-992.
32. Mahmoud A,M.Nawwar, a. Sahar and M.Hussein, *et al*. Phytochemistry, 36(4), 1994, 1035-1037.
33. Djurdjevic l, m.mitrovic, p.avlovic, G.Gajic, and O.ostic, *et al. Arch Environ Contam Toxicol*. 50(4), 2006, 488-495.
34. Gaston b, la grande flore en couleurs.france, Suisse, belgigue et pays voisins, tomc1. E’ditions berlin paris, 1998. 373.

35. Sharma s.k and v.s parmar*, et al*. *j scient and industrial res*. 57, 1998, 873-890.
36. Tagarelli G, A Tagarelli and A.piro, *et al.* Folk Medicine used to heal malaria in Calabria (southern Italy). *j ethnobiol ethnomed.* 6, 2010, 27-29.
37. London sj, yuan jm , and chung F-L, *et al.* Isothiocyanates glutathione-s- transferase m1 and t1 polymorphisms, and lung cancer risk ,a prospective study of men in shanghai, china .lancet. 356 (9231), 2000, 724-729.
38. Sazia Afreen Rahee, Jony Mallik, *et al.* Phytochemical screenings of the methanol extract of whole plant Borreria articulari. *Int Jour Pharm & Bio Arh*. 3(5), 2012, 1062-1066.
39. J.B Harborne Phytochemical Methods. A guide to modern techniques of plant analysis. Springer International Edi. 3rd Edi-1998, Reprint in india 2005.
40. Prashant Tiwari, Bimlesh Kumar, Mandeep Kaur, Gurpreet Kaur, Harleen Kaur, *et al.* Phytochemical screening and Extraction: A Review. *Int Phar Sci*. 1(1), 2011, OECD.425 3 October 2008.
41. Henck j.w, WR Craft, A. Black, J.Colgin and J.A. Anderson, *et al.* Pre and post natal toxicity of the HMG-CoA reductase inhibitors atorvastatin in rats. *Toxi.sci*, 4, 1998, 88-89.
42. Sorg da & Buckner b, A sample method of obtaining venous blood from small laboratory animals. *proced soc Exp.biol.med*, 115, (1964), 1131.
43. Vogel wh, Scholkens ba sandow J, and muller G, *et al.* Drug discovery and evaluation. 2ND ed. New York. *Springer-verlog berlin hiedelberg*, 2002, 1103- 1104.
44. Friedewald wt, levy r.i & fredrickson ds. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *clin chem*. 18, 1972, 499-502.
45. Bucolo G and David M, *et al.* Enzymatic colorimetric test for triglycerides with lipid clearing factor. *Clin chem.* 19, 1973, 476.
46. Naden R, Bachoris PS & Alber JJ, *et al.* Lipids, lipoproteins and apolipoproteins. Tietz fundamentals of clinical chemistry. Philadelphia USA 2001, Ed 5,4.
47. Bangham AD and Home RW, *et al.* Action of saponins on biological membranes, *Nature,* 1962, 952- 953.
48. Ayinla MT, Dada SO, Shittu ST, Olayaki LA, Akiode AO and Ojulari SL, *et al.* Anti-hyperlipidemic effect of aqueous leaf extract of Ocimum gratissimum in alloxan induced diabetic rats. *International Journal of Medicine and Medical Sciences*. 3(12), 2011, 360-363.
49. Bottcher H, Bachorik P.S, Denke M.A, Stein E.A and Rifkind, *et al.* B.M: Lipid and Dyslipoproteinemia, In Clinical Diagnosis and Management Miracle drugs, Zagreb: Zora; 1965, 23–139.
50. Ahmed S.M, Clasen M.D, and Donnelly, *et al.* M.D: Management of dyslipidemia in adults, American Family Physician, 57(9), 1998, 1-16.
51. American Heart Association: Scientific statement on, Cholesterol and Hyperlipidemia, 2005, 1- 2.
52. Aminoff M.J, Tjerney L.M, Nervous system, *et al.* In Current Medical Diagnosis and Treatment, The McGraw-Hill company, 43rd edition, 2004, 956- 963.
53. Abderrahmane Aissaoui, Soumia Zizi, Zafar H, *et al.* Israili, Badiaa Lyoussi, Hypoglycemic and hypolipidemic effects of coriandrum sativum L. in Meriones shawi rats, *Journal of Ethanopharmacology* 137, 2011, 652-661.
54. A Trovato, M.T. Monforte, R. Barbera, A. Rossitto, E.M. Galati, A.M. Forestieri, et al. Effects of fruit juices of Citrus sinensis L. and Citrus limon L. on experimental hypercholesterolemia in the rat, Phytomedicine. 2 (3), 1996, 221-227.
55. Akila M, Devaraj H. Synergistic effect of tincture of crataegus and Mangiferaindica L. Extract on hyperlipidemic and antioxidant status in atherogenic rats. VascPharmacol. 49, 2008, 173-177.