

## Research Article

### Cardioprotective Effect of *Commiphora Wightii* (Guggul) in High Fat Diet Induced Hyperlipidemic Rats

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**Abstract:** Cardioprotective role of *Commiphora wightii* (guggul) were investigated by monitoring biochemical parameters in high fat diet induced hyperlipidemic male albino rats. The hyperlipidemic rats co-administered with 400mg/kg bw ethyl acetate extract of *Commiphora wightii* resins (EACWR) for 45 days. Enzymatic, non-enzymatic antioxidants, lipid peroxidation, protein oxidation, nitrate and inflammatory markers (CRP, COX, XO) were investigated. Increased level of lipid peroxidation, protein oxidation, XO, CRP and COX whereas decreased level of SOD, CAT, GSH, GPx, GR, nitrate level were observed in cardiac tissues of hyperlipidemic rats whereas restoration of above parameters towards normalcy were found in EACWR treated hyperlipidemic rats. Improved endothelial lining and cardiac function is attributed to enhance NO bioavailability due to EACWR treatment and represents a pharmacological approach to prevent major complications associated with oxidative burden and inflammation as a result of hyperlipidemia in cardiovascular disorders.

**Keywords:** Hyperlipidemia, Oxidative stress, Inflammation, Cardiac disorders, *Commiphora wightii*

#### INTRODUCTION

Endothelial dysfunction has been associated with early pathological event of hypercholesterolemia, hypertension, diabetes, ageing and atherosclerosis [1]. It is characterized by a shift in the actions of the endothelium towards impaired modulation of vascular growth, altered anticoagulant and anti-inflammatory activity [2].

Large body of scientific research have illustrated that hyperlipidemia is coupled with oxidative/nitrosative stress, injuring the myocardium and endothelium system [3,4]. Hyperlipidemia induced by high cholesterol diet induces free radicals enormously such as reactive oxygen species (ROS), consequently leads to upsurge in oxidative burden which outstrips endogenous antioxidant defense mechanism [5]. Reactive oxygen species (ROS) oxidize low density lipoprotein (LDL), causes decrease in nitric oxide synthase (NOs) activity [6]. Endothelium-derived nitric oxide (NO) is thought to contribute as vasodilator, anti-inflammatory and anti-proliferative role which improves endothelial function and regresses atherosclerosis [7].

Peroxy nitrite, a cytotoxic product of superoxide anion and nitric oxide reaction generates in the myocardium as result of oxidative stress. Peroxy nitrite has been known to damage DNA, to increase lipid peroxidation, modify various protein post-translationally [8,9]. Moreover, antioxidants released by vascular endothelial lining decrease cellular production of ROS, and reduce endothelial dysfunction, and up regulates the biological activity of endothelium-derived nitric oxide [10].

In this regard, herbal antioxidants (oxygen radical scavengers) well known to improve cardiovascular functions by suppressing oxidative and inflammatory modification mediated by high fat diet [11,12].

*Commiphora wightii* (family: Burseraceae) commonly known as *Guggul*, a revered herb in ayurveda. Is widely found in different geographical regions of India, Bangladesh, and Pakistan [13, 14]. *Commiphora wightii* contain abundant flavonoids such as E- and Z- isomers of guggulsterone, quercetin, beta-sitosterol, myricyl alcohol, amino acids, myrcene and caryophyllene [15]. These flavonoids have been employed as pharmacotherapeutics to treat variety of diseases including hypercholesterolemia, atherosclerosis, diabetes, hypertension, and obesity [16]. The anti-oxidative potential of *Commiphora wightii* containing bioactive compound guggulsterone has been reported to prevent oxidation of LDL and production of free oxygen radicals *in vitro* [17]. Both isomers of guggulsterone (Z and E) demonstrated cardioprotective and antioxidative properties where Z-isomer was more potent than E- isomer in mediating these effects [18]. Neuroprotective effects against oxidative stress had been proved with increased levels of reduced glutathione (GSH) in guggulipid treated rats [19]. In a study, guggulsterone reversed isoproterenol induced oxidative stress in cardiac tissues [20]. Guggulsterone, antioxidative property was confirmed by decreased levels of creatine phosphokinase, phospholipase, and xanthine oxidase, low levels of lipid peroxides and superoxide dismutase [21].

With a large body of scientific evidence we aimed to investigate the effect of ethyl acetate extract of

*Commiphora wightii* resins (EACWR) on overall improvement to alleviate the detrimental outcomes associated with high fat diet induced hyperlipidemia on endothelial dysfunctions in rats.

## MATERIAL AND METHODS

### Chemicals

Nitroblue tetrazolium Cat N-5514 (NBT), thiobarbituric acid Cat T-5500 (TBA), phenazinemetosulphate Cat N-9625 (PMS), nicotinamide adenine dinucleotide Cat N-6754 (NADH), 5,5'-dithio bis 2- nitrobenzoic acid Cat D-5420 (DTNB), nicotinamide adenine dinucleotide phosphate Cat N- 7785 (NADPH) trichloroacetic acid Cat T- 8657 (TCA) and reduced glutathione Cat G-4251 (GSH) were purchased from Sigma Chemical Co., St. Louis, MO, USA. All other reagents used were of high quality and analytical grade.

### Extract preparation

Gum resins of *Commiphora wightii* (Arn.) Bhandari was collected locally in late October, 2011. The plant was taken to the laboratory and was authenticated. Gum-resin of guggul was extensively washed under tap water, followed by washing with sterilized distilled water. They were further air-dried on filter paper at room temperature and then powdered with the help of sterilized pestle and mortar under aseptic condition. Air-dried powder (10g) of the resins was thoroughly mixed with 100ml organic solvent (ethyl acetate). The mixture was placed at room temperature for 24 h on orbital shaker at 150 rpm. Solution was filtered through muslin cloth and then re-filtered by passing through Whatman's Filter No.1. The filtrate thus obtained was concentrated by complete evaporation of solvent at room temperature (25°C) to yield the pure extracts. Stock solutions of crude guggul extracts were prepared by mixing well the appropriate amount of dried extracts and the ethyl acetate solvent to obtain a final concentration of 100mg/ml. Then solution was stored in refrigerator 4°C after collecting in sterilized bottles until further used.

### Study design

Thirty two healthy male albino wistar rats were used in this study each 150-300g body weight. The rats were housed in polypropylene cages separately under standard conditions (12 h light and dark cycles, at 25±30°C and 35-60% humidity). The study was approved by Institutional Animal Ethical Committee, NIMS University, Jaipur, Rajasthan, India.

### Preparation of hyperlipidemic diet

High fat diet includes following ingredients: Wheat flour 45%, sucrose 20%, casein 20%, coconut oil 10%, salt mixture 4.0%, vitamin mixture 1.0%, Cholesterol 1%, cholic acid 1%.

### Different group of animals

Animals were divided into four groups (n=8): control(CON) received normal diet ad libitum,

hyperlipidemic rats (HL) received high fat diet, control treated with ethyl acetate extract of *C. wightii* resins (CON+EACWR) and hyperlipidemic rats treated with ethyl acetate extract of *C. wightii* resins (HL+EACWR) at dose 400mg/kg bw for 45 days. In a daily routine a known amount of fresh pellets were replenished and also food consumed on the previous day was quantified. Body weight of animals in each group was recorded twice in a week to investigate the percentage of weight gain. After sixty days, the animals were fasted overnight, sacrificed by anaesthetic overdose of sodium pentobarbitol (40mg/kg) and blood was withdrawn by cardiac puncture, allowed to clot for 45min and serum was separated by centrifugation at 2500rpm for 10 min. immediately the heart was dissected out and washed with ice-cold saline. Ten percent homogenate of tissue was prepared by using tissue homogenizer at 4°C in 0.1M phosphate buffer.

### Phytochemical analysis of plant extracts

Phytochemical analysis of the ethyl acetate extracts of resins of *Commiphora wightii* was carried out [22, 23] to isolate active phytoconstituents responsible for hypolipidemic effect. Phytochemical evaluation data showed the presence of Z-guggulsterone, E-guggulsterone, terpenes, myrcene, eugenol, phenylpropanoids.

### Oxidative stress markers

Lipid lipid peroxide (LPx) levels were measured by the method of Okhawa et al [24]. The thiobarbituric acid reacting substances (TBARS) of the sample were estimated spectrophotometrically at 532 nm. Superoxide dismutase (SOD EC 1: 15.1.1) activity was determined from its ability to inhibit the reduction of NBT in presence of PMS according to the method of McChord and Fridovich [25] and its activity was expressed as U/mg protein (1 unit is the amount of enzyme that inhibit the reduction of NBT by one half in above reaction mixture). Catalase(CAT, EC 1.11.1.6) activity was assayed as per the method of Aebi et al [26] using hydrogen peroxide as substrate; the decomposition of H<sub>2</sub>O<sub>2</sub> was followed at 240nm on spectrophotometer. It is expressed in µmol of H<sub>2</sub>O<sub>2</sub> consumed/min/mg protein. Glutathione peroxidase (GSHPx, EC 1.11.1.0) was assayed by the method of Pagila and Valentine [27]. It is expressed in µg GSH consumed/ min/mg protein Reduced glutathione (GSH) content was measured using Ellman reagent (5, 5' dithiobis (2-nitro benzoic acid) by the method of Ellman et al, [28]. The optical density of the pale colour was measured on the spectrophotometer on 412 nm. Glutathione reductase (GR) activity (EC.1.6.4.2, GR) activity was assayed by Haselton [29].

### Assessment of inflammatory markers

Heart homogenate was used for quantifying cytosolic xanthine oxidase (XO) activity [30], cyclooxygenase (COX) by using EIA kit (Cayman Chem., Ann Arbor, MI, USA), C-reactive protein

(CRP) activity was done by the separated plasma was assayed for C-reactive protein by latex agglutination method using Accucare creactive protein reagent latex test diagnostic kit (Labcare Diagnostics India Pvt Ltd) [31]. The serum concentration of nitrate (a metabolite of NO) was measured to estimate NO production, reaction catalysed by eNOs (commercially available kit: Biooxytech nitric oxide, USA).

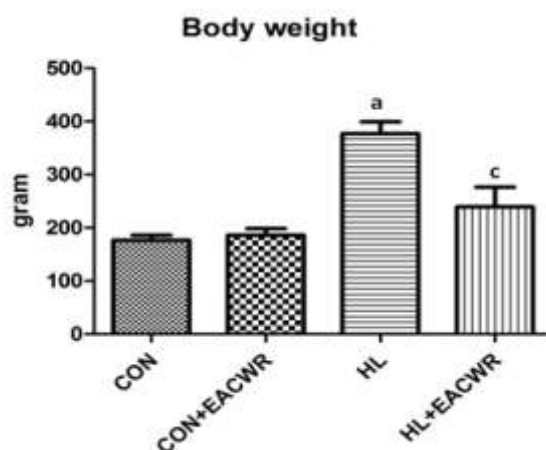
**Statistical analysis**

Results are expressed as Mean±SEM and subjected to one-way analysis of variance (ANOVA)

followed by Student Newman-Keuls post hoc test and values with *p*<0.05 were considered to be statistically significant. InStat (version 3) was used for analysis of data.

**RESULTS**

Figure-1 shown in the study also demonstrated that high fat diet have significantly (*p*<0.001) increased in body weight of HL rats treated with high fat diet. Whereas HL group significantly (*p*<0.01) decreased the body weight when administered with EACWR.



**Figure-1 Effect of EACWR on body weight.**

Values are expressed as Mean±SEM, n=8 animals in each group. Comparisons were made between: a, group CON vs. HL and b, group HL vs. HL+EACWR and c, group CON vs CON+EACWR. Superscript symbols represent statistical significance: *P*<0.05.

Table-1 depicts the consumption of high fat diet significantly decreased activities of enzymatic anti-oxidants i.e. SOD (*p*<0.05), CAT (*p*<0.01), GPx, GR, GST (*p*<0.001) as well as non enzymatic anti-oxidants i.e. GSH (*p*<0.001) were measured in the heart of HL group of rats were as compared to CON group. Restoration of all above parameters near to control were

recorded on treatment with (EACWR 400mg/kg bw) in hyperlipidemic rats. There were statistically significant increment in SOD, CAT, GR, GST (*p*<0.05) whereas GPx (*p*<0.001) and GSH (*p*<0.01) in CON+ EACWR. In case of CON treated with EACWR group indicated increased antioxidants level but statistically nonsignificant except CAT (*p*<0.05).

**Table-1 Effect of EACWR on oxidative stress in cardiac tissues of Control and hyperlipidemic rats**

Parameter	CON	CON+EACWR	HL	HL+EACWR
LPO	7.95 ± 0.29	6.87 ± 0.31	14.4 ± 0.46 <sup>a</sup>	11.8 ± 0.28 <sup>b</sup>
PC	3.82 ± 0.19	3.22 ± 0.85	6.43 ± 0.22 <sup>a</sup>	4.69 ± 0.26 <sup>b</sup>
SOD	27.34 ± 2.13	30.5 ± 1.71	18.4 ± 2.48 <sup>a</sup>	25.9 ± 2.56 <sup>b</sup>
XOD	20.57 ± 1.97	17.9 ± 1.94	32.3 ± 2.01 <sup>a</sup>	26.1 ± 1.6 <sup>b</sup>
CAT	22.10 ± 2.53	29.01 ± 2.3	11.0 ± 0.61 <sup>a</sup>	19.67±7.9 <sup>b</sup>
GPx	5.11 ± 0.13	5.15 ± 0.14	4.15 ± 0.13 <sup>a</sup>	5.04 ± 0.19 <sup>b</sup>
GR	30.72 ± 1.13	32.7 ± 1.3	23.7 ± 0.85 <sup>a</sup>	27.7 ± 0.91 <sup>b</sup>

Values are expressed as Mean±SEM, n=8 animals in each group. Comparisons were made between: a, group CON vs. HL and b, group HL vs. HL+EACWR and c, group CON vs CON+EACWR. Superscript symbols represent statistical significance: *P*<0.05

High fat diet significantly increased lipid peroxidation rate ( $p < 0.001$ ) in HL group of rats as compared to CON group. Whereas, HL and CON group treated with EACWR are exhibited significant decreased MDA level ( $p < 0.001$  and  $p < 0.05$  respectively).

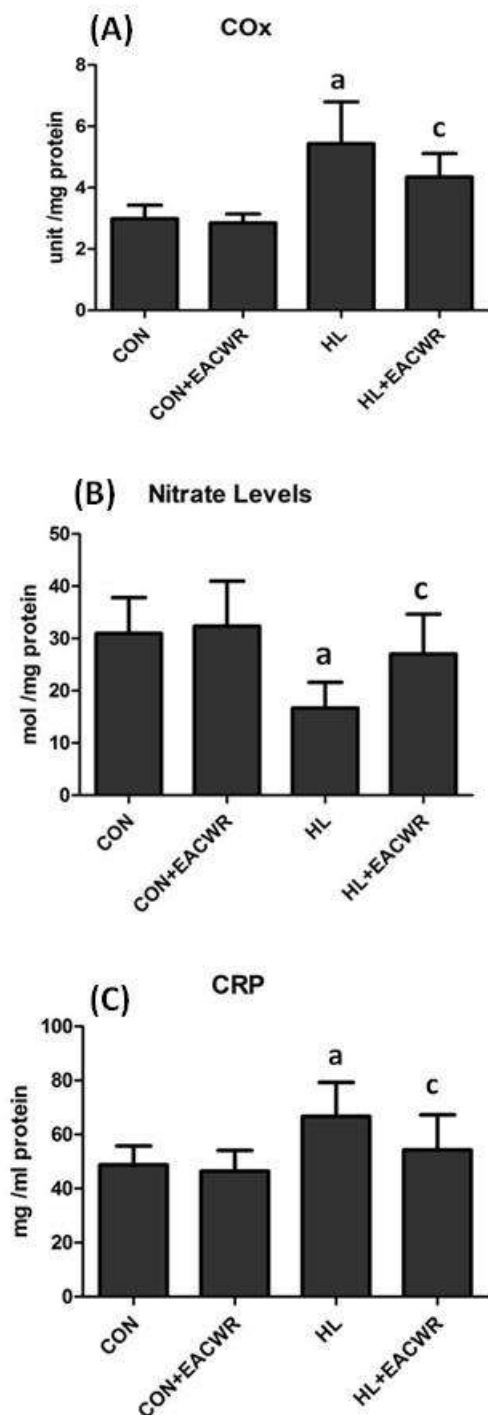


Fig-2 - Effect of EACWR on COX, Nitrate and CRP levels

Values are expressed as Mean±SEM, n=8 animals in each group. Comparisons were made between: a between: a, group CON vs. HL and c, group HL vs. HL+EACWR. Superscript symbols represent statistical significance:  $P < 0.05$ .

In present study displayed high level of protein oxidation ( $p < 0.001$ ) in HL group of animals on excessive intake of high fat diet as compared to CON group. We observed significantly suppressed ( $p < 0.001$ ) rate of protein oxidation in HL group administered with EACWR. Whereas statistically insignificant ( $p > 0.05$ ) decrease in protein oxidation were displayed by CON group treated with EACWR (figure- 2). Table-1. Hyperlipidemic diet also shows profound effects of inflammation in HL group of rats. Xanthine oxidase activity in heart was markedly increased ( $p < 0.001$ ) in HL group as result of inflammation induced by high fat diet as compared to CON group. On the other side HL group animals treated with EACWR were significantly ( $p < 0.05$ ) recovered from inflammatory effects of xanthine oxidase activity near to control. Whereas cardiac tissues of CON group treated with EACWR showed non significantly ( $p > 0.05$ ) decreased level of xanthine oxidase activity.

Figure-2 shows inflammatory markers, like CRP level in HL group of rats, were significantly ( $p < 0.01$ ) high as compared to CON. It has been found that EACWR administration significantly ( $p < 0.05$ ) regress CRP level in plasma of HL group as compared to HL group rats. Whereas CRP level were non-significant ( $p > 0.05$ ) decreased in CON group treated with EACWR. We observed highly significant ( $p < 0.001$ ) level of COX activity in PBMC cells of HL group of animals as compared to their control counterparts in CON group. Treatment of EACWR to HL group significantly ( $p < 0.001$ ) decreased COX activity in comparison to HL group. Whereas there is insignificant ( $p > 0.05$ ) decrease in COX activity was observed in CON group treated with EACWR.

Comparing with the controls, nitrate level were significantly low ( $p < 0.05$ ) in the plasma of high fat diet treated rats. No apparent change in nitrate level ( $p > 0.05$ ) was measured in CON group rats treated with EACWR. In HL group animals fed with EACWR ( $p < 0.05$ ), the declined nitrate level was returned near to CON group animals (figure-3).

## DISCUSSION

In HL group of rats, the decrease in the enzymatic and non enzymatic (SOD, CAT, GPx, GR, and GSH) antioxidants could be due to a feedback inhibition or oxidative inactivation of the enzyme protein due to increased ROS generation [32]. Many reports also supported our result that due to high fat induction there was weakened free radical scavenging action which contributes to the reduced antioxidants in HL group [33]. A study have indicated that high fat diet can also modify antioxidant activities by suppressing functional sulfhydryl (SH) groups present in several enzymes such as SOD, CAT [34].

HL group animals administered with EACWR restored the altered antioxidants near to control group. There is large pool of evidence which illustrate one of the several polyphenol compounds isolated from the guggul is guggulsterone, possess strong antioxidant

properties, which reduce the formation of ROS by directly inhibiting the reactive oxygen generating enzymes [35]. It has been suggested that antioxidant nature of guggulsterols could be due to presence of hydroxyl groups at  $\alpha$ -positions of double bonds, and its soluble in lipids. Their structure is similar to antioxidant vitamins. The steroid structure also contains H, CH<sub>3</sub> and O bond, which possess free radicals such as hydroxyl and singlet oxygen quenching property like other herbal drugs. It was demonstrated that guggulipid, the resin of *C. wightii* declined rate of lipid peroxidation in hypercholesterolemic humans [36]. Indeed, results of present study also shown significant reduction in the formation of TBARS and protein carbonyl content in parallel with improvement in the levels of both enzymatic and non-enzymatic antioxidants in cardiac tissues.

In accordance with previous studies, present finding states that high fat diet leads to decreased nitrate level. It depicts low level of cardiac nitric oxide (NO), this could be due to diminished NO biosynthesis or increased NO degradation. It is interesting to speculate that hypercholesterolemia induces excess amount of superoxide anion reacts with nitric oxide, potent vasodilator and forms a toxic product peroxynitrite, consequently perform endothelial dysfunction [37]. Superoxide anion may be found abundantly due to depletion of enzymatic and nonenzymatic antioxidants in cardiac tissue of HL group. One of the major sources of superoxide anion in the rat heart is xanthine oxidase. HL group shows increased level of xanthine oxidase activity. In HL group treated with EACWR reduced the xanthine oxidase activity, this present study is in agreement with Chander *et al.*, [35].

Elevated cholesterol has also been shown to trigger the release of the inflammatory mediator like C-reactive protein (CRP), a useful clinical marker of CVD. It is pellucid to say that CRP, via IL-8, may exacerbate vascular dysfunction by inhibiting eNOS [38, 39]. It can be suggested with present set of data that administration of EACWR in HL group has rejuvenated endothelial functions.

Prostaglandin-H<sub>2</sub>-synthase or cyclooxygenase (COX) is a rate-limiting enzyme in the biosynthesis of prostaglandins (PG). COX enzymes are crucial bodies in the inflammatory events. Prostaglandin (PG) biosynthesis has been responsible in the pathophysiology of cardiovascular processes and a range of inflammatory diseases [40]. In the present study, supplementation with ethyl acetate extract of *Commiphora wightii* resins (EACWR) decrease the activity of COX in the monocyte extracted from hyperlipidemic rats, it could be due presence of active compound guggulsterone [41]. According to scientific data, standard alcoholic fraction of guggulipid containing E-guggulsterone, proved to be free radical scavenger and protected skin against superoxide anions and hydroxyl radicals in non-enzymatic test systems [25]. These results suggest that EACWR gives shielding

effects against the inflammation induced by high fat diet in HL group of rats.

Oleoresin of *C. wightii* consist of numerous steroid lipids. Z-guggulsterone and E-guggulsterone are the most effective steroids [42]. Moreover, the isomeric structure of guggulsterols is similar to tocopherols and exhibit least toxicity to body due to absence of highly reactive groups. In preclinical as well as clinical studies, guggulipid and guggulsterone have been demonstrated to regress the risk in the progression of cardiac events [43-45]. Thus, the antioxidant and anti-inflammatory role of *C. wightii* established in the present study may be due to the phytochemical component, guggulsterone of the resin.

## CONCLUSION

The results of the present study suggest that *C. wightii* provides significant protection to vascular endothelial system and cardiac tissues against the consequence of hypercholesterolemia induced oxidative stress and inflammation by scavenging reactive oxygen species and blocking free radical interactions with biomolecules. Thus, ethyl acetate extract of *C. wightii* resins (400mg/kg bw) may potentially beneficial in prevention of hypercholesterolemia induced cardiovascular diseases. However, further clinical studies are required to assess the efficacy and safety of *C. wightii* in hyperlipidemic humans in order to elucidate the fundamental mechanism of action of the active ingredient of *C. wightii* resins that mediates the protective effect.

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