Evaluation of lipid- lowering and antioxident properties of methanolic extract of leaves of *Anogeissus latifolia wall* on high fed cholestrol and triton induced models in Wister albino rats

Shaik Eesuf Pasha*, M.Janarthan, Shaik Mallika, MD.Akbar
*Nimra College of Pharmacy, Ibrahimpatnam, Vijayawada, Andhra Pradesh, India
***Corresponding author: E.Mail: eusaf.pasha@gmail.com**

ABSTRACT

Lipid lowering and antioxidant properties of methanolic extract of leaves of *Anogeissus latifolia* Wall (MEALL) was studied on high fed cholesterol and triton induced models in Wistar albino rats. The results were satisfactory. Methonalic extract of *Anogeissus latifolia* was significantly reduced total cholesterol, triglycerides, LDL and VLDL levels as well as increased HDL level in high fed cholesterol and Triton X 100 (TR) induced hyperlipidemic models. Also, the atherogenic index of Methonalic extract of Anogeissus latifolia treated groups showed was significant reduction compared to hyperlipidemic control.

KEY WORDS: Lipid lowering, antioxidant properties, *Anogeissus latifolia.*

1. INTRODUCTION

Diabetes, obesity, chronic renal diseases, primary hyperlipoproteinemia is carrying high risk of cardiovascular disorders. Hypertriglyceridemia in combination with abnormally low concentration of HDL cholesterol is one of the most common and atherogenic profile of lipid metabolism of high prevalence seen in populations. Investigation of natural products is gaining importance and demand to use natural products in treatment of dyslipidemia is increasing worldwide. Scientific scurtinity on natural products might lead to the development of alternative drugs and it may help to manage dyslipidemia effectively.

Anogesious latifollia (combartacea) is widely distributed in India and used ethnically by tribal people to control blood sugar. Its leaves and bark possess blood glucose lowering effect in diabetic condition. Current literature search revealed that, till date, the lipid profile lowering activity of *Anogesisus latifollia* leaves are not studied in high fat diet and triton induced hyperlipidemic animal models. Hence, present study was aimed to comparative evaluation of lipid lowering and antioxidant potential of methanol extract of the *Anogesisus latifollia* leaves an in high dose fed cholesterol and triton induced hyperlipidemic animal models to document its efficacy.

2. MATERIALS AND METHODS

Collection of plant material: Leaves of the *Anogeissus latifolia* was collected near from Kondapalli in Vijayawada, Andhra Pradesh. The leaves was authenticated by Dr.P.Satyanarayana Raju in Acharya Nagarjuna University, Guntur, Andhra Pradesh. The root was separated from adulterants, shade dried and powdered coarsely. It was packed in air-tight container up to the completion of study.

Extraction of plant material: About 80 g of air dried powdered plant materials was taken in Soxhlet apparatus and extracted with petroleum ether for up to discoloration of solution. After 72 h, the powder was taken out and dried. Then it was packed again and extracted with methanol till the colour disappeared. The methanolic extract of *Anogeissus lotifolia* leaves concentrated under reduced pressure using rota-evaporator. The concentrated extract was stored in refrigerator at 10°C up to the completion of pharmacological studies.

Experimental animals: Male wistar albino rats (150-200 g) were used for the antihyperlipidemia studies. Female swiss albino mice (25-30 g) were used for the acute toxicity study. The animals were obtained from Mahaveer Enterprices, Medipalli, Board Uppal Mandal, Hyderabad. All animals were housed for at least one week in the laboratory animal room prior to study. The selected animals were housed in polypropylene cages in the standard environmental conditions (20-25°C), 12:12 light: dark cycle, fed with standard rodent diet and water *ad libitum*. The experiments on animals were conducted in accordance with the international accepted principle for laboratory animal use and the experimental protocols duly approved (005/NCP/IAEC/2015) by the institutional animal ethical committee (IAEC) of NIMRA college of pharmacy Ibrahimpatnam, Vijayawada.

Methanolic extract of *Anogeisuss latifolia* dose selection: Dose selecton was done by previous review of literature.

The methanolic extract of *Anogeissus latifolia* was dissolved in water and administered to the respective group of animals throughout the experiment. Each day methonalic extract of *Anogeissus latifolia* were prepared freshly before administration.

Preliminary phytochemical analysis: Test for carbohydrates, alkaloids, streroids &sterols, glycosides, saponins, flavonoids, tannins, protein and amino acids, phenols and terpenoids were conducted.

Experimental design for High dose cholesterol induced model for antihyperlipedemic activity: The rats were divided into five groups.

Group 1: Normal control group received normal saline (5 ml/kg).

Group 2: Hyperlipidemic group received cholesterol (25 mg/kg).

Group 3: Test group received methonalic extract of *Anogeissus latifolia* (100 mg/kg) along with cholesterol

Group 4: Test group received methonalic extract of *Anogeissus latifolia* (200 mg/kg) along with cholesterol

Group 5: Positive control group received atorvastatin (10 mg/kg) along with cholesterol

The above groups 2 to 5 were fed cholesterol in coconut oil orally at 10 am and methonalic extract of *Anogeissus latifolia* and atorvastatin was given orally at 4 pm daily to respective groups for period 30 days. The normal control group was treated with normal saline and standard laboratory was provided to all groups. The body weight of all group were taken weekly. On 15th and 30th day, rats were fasted overnight and anaesthetized with ketamine (100 mg/kg, i.p.); blood sample was collected through retro-orbital plexus puncture to the respective group animals for the biochemical parameters estimation. Blood samples were kept at room temperature for 30 minutes and serum was separated by centrifugation at 10000 rpm for 10 min. On 30th day animals after the blood collection, all group animals were sacrificed by cervical dislocation, kidney and liver were isolated and washed with normal saline for *in vivo* antioxidant levels.

Experimental design for Triton model: The rats were divided into five groups.

Group 1: Administered vehicle and served as normal control.

Group 2: Administered Triton X 100 (TR) (100mg/kg) and served as TR control.

Group 3: Test group received Methanolic extract of *Anogeissus latifolia* (100 mg/kg) along with TR (100mg/kg).

Group 4: Test group received Methanolic extract of *Anogeissus latifolia* (200 mg/kg) along with TR (100mg/kg).

Group 5: Positive control group received atrovastatin (10 mg/kg) along with TR (100mg/kg).

Forty two male Wistar rates weighing 150 to 200 grams were randomly divided into 5 groups of each. The above groups 2 to 5 animals were starved for 18h and, then TR at a dose of (100mg /kg) of body weight was given through i.p. route. After 20 hours and 42 hours the MEALL at a dose of 100 and 200 mg/kg was given through oral route to their respective groups. The control group received only normal saline. At 22nd and 44th hour the animals were anaesthetized with ketamine (100 mg/kg, i.p.) and blood sample was collected through retro-orbital plexus puncture to the respective group animals for the biochemical parameters estimation. Blood samples were kept at room temperature for 30 minutes and serum was separated by centrifugation at 10000 rpm for 10 minutes.

3. RESULTS AND DISCUSSION

Preliminary phytochemical analysis: The preliminary photochemical analysis of Methonalic extract of *Anogeissus latifolia* showed the presence of carbohydrates, alkaloids, streroids &sterols, glycosides, saponins, flavonoids, tannins, phenols and terpenoids were conducted and absence of Proteins & amino acids

Table.1.Preliminary photochemical analysis of Methonalic extract of *Anogeissus latifolia*

Photochemical constituents	Methonalic extract of *Anogeissus latifolia*
Carbohydrates	+ve
Alkaloids	+ve
Steroids & sterols	+ve
Glycosides	+ve
Saponins	+ve
Flavonoids	+ve
Tannins	+ve
Proteins & amino acids	-ve
Phenols	+ve
Terpenoids	+ve

High dose cholesterol induced model:

Effect of MEALL on lipid profiles:

Total cholesterol (TC): The oral administration of cholesterol to the rats showed significant ($P < 0.001$) increased total cholesterol level at 15 and 30 day in hyperlipidemic control compared to normal control. In hyperlipidemic rats, administration of Methonalic extract of *Anogeissus latifolia* (100 and 200 mg/kg) and atorvastatin (10 mg/kg) significantly ($P < 0.001$) reduced TC levels than hyperlipidemic control. The total cholesterol reduction efficacy of Methonalic

extract of *Anogeissus latifolia* 100 and 200 mg/kg was significant higher ($P < 0.001$) when compared to Methonalic extract of *Anogeissus latifolia* (100 mg/kg) on 15th and 30th day.

Triglycerides (TG): The oral administration of cholesterol to the rats showed significant ($P < 0.001$) increased TG level at 15 and 30 day in hyperlipidemic control compared to normal control. In hyperlipidemic rats, administration of Methonalic extract of *Anogeissus latifolia* (100 and 200 mg/kg) and atrovastatin (10 mg/kg) significantly ($P < 0.001$) and Methonalic extract of *Anogeissus latifolia* (200 mg/kg) significantly ($P < 0.05$) reduced TG levels than hyperlipidemic control.

High Density Lipoprotein (HDL): The oral administration of cholesterol to the rats showed significant ($P < 0.001$) decreased HDL level at 15 and 30 day in hyperlipidemic control compared to normal control. In hyperlipidemic rats, administration of Methonalic extract of *Anogeissus latifolia* (100 and 200 mg/kg) and atrovastatin (10 mg/kg) significantly ($P < 0.001$) increased HDL levels than hyperlipidemic control.

Low Density Lipoprotein (LDL): The oral administration of cholesterol to the rats showed significant ($P < 0.001$) increased LDL level at 15 and 30 day in hyperlipidemic control compared to normal control. In hyperlipidemic rats, administration of Methonalic extract of *Anogeissus latifolia* (100 and 200 mg/kg) and atrovastatin (10 mg/kg) significantly ($P < 0.001$) reduced LDL levels than hyperlipidemic control. The LDL reduction efficacy of Methonalic extract of *Anogeissus latifolia* (200 mg/kg) was significant higher ($P < 0.001$) when compared to Methonalic extract of *Anogeissus latifolia* 100 on 30 day. The data's are given in Table 16 and graphical representation given in Figure 8.

Very Low Density Lipoprotein (VLDL): The oral administration of cholesterol to the rats showed significant ($P < 0.001$) increased VLDL level at 15 and 30 day in hyperlipidemic control compared to normal control In hyperlipidemic rats, administration of Methonalic extract of *Anogeissus latifolia* (100 and 200 mg/kg) and atrovastatin(10 mg/kg) significantly ($P < 0.001$) reduced VLDL levels than hyperlipidemic control on 30 day. **Atherogenic Index (A.I):** The oral administration of cholesterol to the rats showed significant ($P < 0.001$) increased A.I level at 15th and 30th day in hyperlipidemic control compared to normal control. In hyperlipidemic rats, administration of Methonalic extract of *Anogeissus latifolia* (100 and 200 mg/kg) and atrovastatin (10 mg/kg) significantly ($P < 0.001$) reduced AI levels than hyperlipidemic control.

Effect of MEALL on body weight: In this study, administration of cholesterol significantly ($P<0.001$) increased body weight in hyperlipidemic control animals compared to normal control animals. Administration of Methonalic extract of *Anogeissus latifolia,* (100 and 200 mg/kg) and atrovastatin (10 mg/kg) significantly ($P < 0.001$) reduced body weight than hyperlipidemic control on 14th, 21th and 28th day.

Serum Glutamate Oxalo Acetate Transaminase (SGOT): In this study, administration of cholesterol significantly ($P<0.001$) increased SGOT levels in hyperlipidemic control animals compared to normal control animals. Administration of Methonalic extract of *Anogeissus latifolia* (200 mg/kg), and atrovastatin(10 mg/kg) significantly ($P < 0.001$) and Methonalic extract of *Anogeissus latifolia* (100 mg/kg) significantly ($P<0.01$) reduced SGOT than hyperlipidemic control.

Serum Glutamate Pyruvate Transaminase (SGPT): In this study, administration of cholesterol significantly ($P<0.001$) increased SGPT levels in hyperlipidemic control animals compared to normal control animals. Administration of Methonalic extract of *Anogeissus latifolia* (100 and 200 mg/kg) and atrovastatin (10 mg/kg) significantly ($P < 0.001$) reduced SGPT than hyperlipidemic control.

Alkaline Phosphate (ALP): In this study, administration of cholesterol significantly ($P<0.001$) increased ALP levels in hyperlipidemiccontrol animals compared to normal control animals. Administration of Methonalic extract of *Anogeissus latifolia* (200 mg/kg) significantly *(*$P<0.001$) and Methonalic extract of *Anogeissus latifolia* (100 mg/kg)atrovastatin (10mg/kg) significantly lower ($P<0.01$) reduced ALP than hyperlipidemic control. ALP reduction efficacy of Methonalic extract of *Anogeissus latifolia* (200 mg/kg) was significant higher ($P < 0.001$) when compared to Methonalic extract of *Anogeissus latifolia* (100 mg/kg).

Creatinine: In this study, administration of cholesterol significantly ($P<0.001$) increased Creatinine levels in hyperlipidemic control animals compared to normal control animals. Administration of Methonalic extract of *Anogeissus latifolia* (200 mg/kg), atrovastatin (10mg/kg) significantly ($P<0.001$) and Methonalic extract of *Anogeissus latifolia* (100 mg/kg), significantly ($P<0.01$) reduced Creatinine level than hyperlipidemic control.

***In vivo* anti-oxidant:**

Catalase in liver: In this study, administration of cholesterol significantly ($P<0.001$) decreased catalase levels in liver in hyperlipidemic control animals compared to normal control animals. Administration of Methonalic extract of *Anogeissus latifolia* (100 and 200 mg/kg), and atrovastatin (10mg/kg) significantly ($P<0.001$) increased catalase level in liver than hyperlipidemic control.

Superoxide Dismutase (SOD) in liver: In this study, administration of cholesterol significantly (*P*<0.001) decreased SOD levels in liver in hyperlipidemic control animals compared to normal control animals. Administration of Methonalic extract of *Anogeissus latifolia* (100 and 200 mg/kg), significantly (*P*<0.001) atrovastatin (10mg/kg) significantly *(P*<0.05) increased SOD level in liver than hyperlipidemic control.

Glutathione Peroxidase (GPx) in liver: In this study, administration of cholesterol significantly (*P*<0.001) decreased GPx levels in liver in hyperlipidemic control animals compared to normal control animals. Administration of Methonalic extract of *Anogeissus latifolia* (100 and 200mg/kg) and atrovastatin (10 mg/kg) significantly (*P*<0.001) increased GPx level than hyperlipidemic control.

Reduced Glutathione (GSH) in liver: In this study, administration of cholesterol significantly (*P*<0.001) decreased GSH levels in liver in hyperlipidemic control animals compared to normal control animals. Administration of Methonalic extract of *Anogeissus latifolia* (100 and 200 mg/kg) and atrovastatin (10 mg/kg) significantly (*P*<0.001) increased GPx level than hyperlipidemic control.

Thiobarbituric Acid Reactive Substances (TBARS) in liver: In this study, administration of cholesterol significantly (*P*<0.001) increased TBARS levels in liver in hyperlipidemic control animals compared to normal control animals. Administration of Methonalic extract of *Anogeissus latifolia* (100 and 200 mg/kg) and atrovastatin (10 mg/kg) significantly.

Catalase in Kidney: In this study, administration of cholesterol significantly (*P*<0.001) decreased catalase levels in kidney in hyperlipidemic control animals compared to normal control animals. Administration of methonalic extract of *Anogeissus latifolia* (100 and 200 mg/kg), and *atrovastatin* (10 mg/kg) significantly (P<0.001) increased catalase level in kidney than hyperlipidemic control.

SOD in kidney: In this study, administration of cholesterol significantly (*P*<0.001) decreased SOD levels in kidney in hyperlipidemic control animals compared to normal control animals. Administration of Methonalic extract of *Anogeissus latifolia* (100 and 200mg/kg) and atrovastatin (10mg/kg) significantly (P<0.001) significantly increased SOD level in kidney than hyperlipidemic control.

GPx in kidney: In this study, administration of cholesterol significantly (*P*<0.001) decreased GPx levels in kidney in hyperlipidemic control animals compared to normal control animals. Administration of Methonalic extract of *Anogeissus latifolia* (100 and 200 mg/kg) and atrovastatin (10 mg/kg) significantly (*P*<0.001) increased GPx level in kidney than hyperlipidemic control.

Reduced Glutathione (GSH) in liver: In this study, administration of cholesterol significantly (*P*<0.001) decreased GSH levels in kidney in hyperlipidemic control animals compared to normal control animals. Administration of Methonalic extract of *Anogeissus latifolia* (100 and 200mg/kg) and atrovastatin (10 mg/kg) significantly *(P*<0.001increased GSH level in kidney than hyperlipidemic control. The increasing efficacy was high in MEALL (200 mg/kg) compared to Methonalic extract of *Anogeissus latifolia* (100 mg/kg).

TBARS in kidney: In this study, administration of cholesterol significantly (*P*<0.001) increased TBARS levels in kidney in hyperlipidemic control animals compared to normal control animals. Administration of Methonalic extrat fo *Anogeissus latifolia* (100 and 200mg/kg) and atorvastatin (10mg/kg) significantly (*P*<0.001) decreased TBARS level in kidney than hyperlipidemic control.

Triton model:

Effect of MEALL on lipid profiles:

AT 22hours: The i.p injection of TR (100mg/kg once) to rats showed significant (*P* < 0.001) increased (TC, TG, VLDL and LDL) and decreased (HDL) all lipid profile at 22 h in TR control compared to normal control. Administration of Methonalic extract of *Anogeissus latifolia* (100 and 200 mg/kg) showed non-significant compared with TR control. Administration atorvastatin (10 mg/kg) significantly (*P* < 0.001) reduced (TC, TG, VLDL and LDL) and decreased (HDL) all lipid profile levels than TR control.

AT 44hours:

Total Cholesterol (TC): The i.p injection of TR (100 mg/kg once) to rats showed significant (*P* < 0.001) increased TC level at 44h in TR control compared to normal control. Administration of Methonalic extract of *Anogeissus latifolia* (200 mg/kg) significant (*P* < 0.001) and Methonalic extract of *Anogeissus latifoli* (100 mg/kg) atrovastatin (10 mg/kg) significant (*P* < 0.01) reduced TC levels than TR control.

Triglycerides (TG): The i.p injection of TR (100mg/kg once) to rats showed significant (*P* < 0.001) increased TG level at 44h in TR control compared to normal control. Administration of Methonalic extract of *Anogeissus latifolia*, (100 and 200 mg/kg) and atorvastatin (10mg/kg) significant (*P* < 0.001) reduced TG levels than TR control.

High Density Lipoprotein (HDL): The i.p injection of TR (100 mg/kg once) to rats showed significant (*P* < 0.001) decreased HDL level at 44h in TR control compared to normal control. Administration of

Methonalic extract of *Anogeissus latifolia* (100 and 200 mg/kg) and atorvastatin (10 mg/kg) significant ($P < 0.001$) increased HDL levels than TR control.

Low Density Lipoprotein (LDL): The i.p injection of TR (100 mg/kg once) to rats showed significant ($P < 0.001$) increased LDL level at 44h in TR control compared to normal control. Administration of Methonalic extract of *Anogeissus latifolia* (200 mg/kg) significant ($P < 0.001$), and atorvastatin (10mg/kg) significant ($P < 0.01$) and MEALL (100 mg/kg) significant ($P < 0.05$) decreased LDL levels than TR control.

Very Low Density Lipoprotein (VLDL): The i.p injection of TR (100 mg/kg once) to rats showed significant ($P < 0.001$) increased VLDL level at 44h in TR control compared to normal control. Administration of Methonalic extract of *Anogeissus latifoli* (100 and 200 mg/kg) and atrovastatin (10 mg/kg) significant ($P < 0.001$) decreased VLDL levels than TR control.

Table.2.Effect of Methonalic extract of *Anogeissus latifolia* on lipid profiles in cholesterol fed induced hyperlipidemic animals

Group	Dose (mg/kg)	TC (mg/dl)		TG (mg/dl)		HDL (mg/dl)		LDL (mg/dl)		VLDL (mg/dl)		Atherogenic index	
		15 Days	30 Days	15 Days	30 Days	15 Days	30 Days	15 Days	30 Days	15 Days	30 Days	15 Days	30 Days
Normal control	Saline (5ml/kg)	59.60 ± 0.99	82.51 ± 0.34	30.75 ± 2.52	119.91 ± 0.85	41.17 ± 0.47	37.01 ± 0.57	13.6 1 ± 0.67	16.33 ± 1.76	6.27 ± 0.98	23.87 ± 0.17	-0.20 ± 0.03	0.50 ± 0.01
Hyperlipidemic control	Cholesterol fed (25mg/kg)	96.6 ± 3.80[a]	113.51 ± 0.59[a]	62.27 ±1.21[a]	173.90 ±1.22[a]	36.33 ±1.48[a]	26.07 ±0.61[a]	45.79 ±4.54[a]	52.60 ± 0.88[a]	12.91 ± 0.17[a]	34.67 ±0.2[a]	0.21 ±0.09[a]	0.84 ±0.01[a]
Methonalic extract of *Anogeissus*	100	56.38 ±0.88[b,e]	84.22 ±0.52[b,e]	47.46 ±0.59[c]	145.00 ± 1.52[b]	53.21 ±0.68[b]	43.00 ±1.15[b]	14.12 ±0.02[b]	14.00 ±0.57[b,g]	9.67 ± 0.08[b]	29.00 ±0.3[b]	0.05 ±0.09[b]	0.52 ±0.08[b]
Methonalic extract of *Anogeissus*	200	47.46 ±0.5[b,f,h]	82.79 ±0.91[b,j]	54.38 ±0.90[c]	131.30 ±0.91[b,h]	54.67 ±0.88[b]	41.27 ±0.73[b]	16.43 ±1.24[b]	15.67 ±0.88[b.h]	11.67 ±0.08[ns]	26.27 ±0.17[b]	0.02 ±0.01[b]	0.48 ±0.12[b]
Atrovastatin	10	74.07 ± 1.71[b]	94.61 ± 2.56[b]	34.08 ±1.16[b]	134.8 ± 1.08[b]	55.00 ± 0.9[b]	42.63 ±0.56[b]	24.36 ±0.15[b]	25.33 ± 1.85[b]	7.23 ±0.31[b]	26.87 ±0.2a[b]	-0.17 ±0.02[b]	0.50 ±0.08[b]

Values are expressed as mean ± S.E.M., (n = 6).

[a]*P*< 0.001 hyperlipidemic control compared with normal control ; [b]*P*< 0.001, [c]*P*< 0.01, [d](P < 0.05 [ns]- Non significant Methonalic extract of *Anogeissus latifolia*, (200mg/kg) and atrovastatin (10 mg/kg) compared with hyperlipidemic control; [e]*P*< 0.001 Methonalic extract of *Anogeissus latifolia* (200 mg/kg) compared with Methonalic extract of *Anogeissus latifolia* (100 mg/kg);

Table.3.Effect of Methonalic extract of *Anogeissus latifolia* on body weight in high cholesterol feed induced hyperlipidemic animals

Groups	Dose (mg/kg)	Body weight (g)				
		Day 0	Day 7	Day 14	Day21	Day28
Control	Saline (5 ml/kg)	171.2 ± 5.68	177.8 ± 5.72	187.8 ± 5.10	191.8 ± 6.93	201.3 ± 6.57
Hyperlipidemic control	Cholesterol fed (25mg/kg)	170.8 ± 4.39	183.0 ± 3.51	199.7 ± 2.90[a]	216.0 ± 3.05[a]	250.0 ± 5.77[a]
Methonalic extract of *Anogeissus latifolia*	*100*	*168.0 ± 5.94*	*180.7 ± 5.20[ns]*	*190.7 ± 6.38[b]*	*201.7 ± 6.09[b]*	*225.0 ± 2.88[b]*
Methonalic extract of *Anogeissus latifolia*	200	169.0 ± 3.68	182.7 ± 4.33[ns]	197.7 ± 3.71[b]	205.0 ± 5.00[b]	211.7 ± 4.41[b]
Atorvastatin	10	176.3 ±4 .08	180.7 ± 5.20[ns]	190.0 ± 5.29[b]	202.7 ± 3.90[b]	213.3 ± 1.66[b]

Values are expressed as ± S.E.M., (n = 6); [a]*P*< 0.001 hyperlipidemic control compared with normal control : [b]*P*< 0.001, ns – non significant Methonalic extract of *Anogeissus latifolia* (100, 200 mg/kg), and atrovastatin (10 mg/kg) compared with hyperlipidemic control

Table.4.Effect of Methonalic extract of *Anogeissus latifolia* on SGOT, SGPT, ALP and creatinine in cholesterol fed hyperlipidemic animals

Group	Dose (mg/kg)	SGOT (U/l)	SGPT (U/l)	ALP (U/l)	Creatinine (mg/dl)
Normal control	Saline (5ml/kg)	117.31 ± 10.08	45.33±2.60	54.67±6.06	0.46±0.03
Hyperlipidemic control	Cholesterol fed (25mg/kg)	239 .02± 2.51[a]	141.01±8.88[a]	188.01±20.82[a]	0.76±0.04[a]
Methonalic extract of *Anogeissus latifolia*	100	156.05±14.18[c]	57.01±5.77[b]	186.02±4.93[ns]	0.53±0.03[c]
Methonalic extract of *Anogeissus latifolia*	200	78.02±12.50[b d e f]	65.67±5.20[b]	96.31±15.45[b d]	0.43±0.03[b]
Atrovastatin	10	165.71±20.63[c]	55.03±5.31[b]	124.05±6.58[c]	0.53±0.03[b]

Values are expressed as S.E.M., (n = 6); [a]P< 0.001 hyperlipidemic control compared with normal control ;.bP< 0.001, cP< 0.01, ns- Non significant Methonalic extract of Anogeissus latifolia (100, 200 mg/kg) and atrovastatin

(10mg/kg) compared with hyperlipidemic control;.[d]P< 0.001 Methonalic extract of *Anogeissus latifolia* (200 mg/kg) compared with Methonalic extract of *Anogeissus latifolia* (100mg/kg)

Table.5.Effect of Methonalic extract of *Anogeissus latifolia* on liver antioxidants in cholesterol fed hyperlipidemic animals

Group	Dose (mg/kg)	CAT (µM of H_2O_2consumed/min/ mg of protéin)	SOD (U/mg of protein)	GPx (µg of GSH consumed/min/ mg protéin)	GSH (µg of GSH consumed/min/ mg protéin)	TBARS (ŋmoles of MDA released /mg protéin)
Normal control	Saline (5ml/kg)	930.61±4.16	101.01±1.26	49.50± 1.00	19.95± 3.19	122.31± 7.36
Hyperlipidemic control	Cholesterol fed (25mg/kg)	372.20±2.42[a]	90.33± 9.15 [a]	24.66± 2.01 [a]	6.89± 0.92 [a]	352.51± 3.68 [a]
Methonalic extract of *Anogeissus latifolia*	100	559.62± 11.69[b]	165.31± 8.68[b]	45.78± 1.38[b]	16.59± 0.55[b]	90.07± 8.33[b]
Methonalic extract of *Anogeissus latifolia*	200	1101.02±3.83[b,e,f]	147.20± 5.31[d]	57.92±2.51[b]	20.44± 0.52[b]	63.93± 7.01[b,e]
Atrovastatin	10	580.32± 4.02[b]	141.11± 1.41[d]	37.68± 0.59[b]	16.89± 0.42[b]	98.43± 4.0[b]

Values are expressed as mean ± S.E.M., (n = 6); [e]*P*< 0.001 hyperlipidemic control compared with normal control. ;[b]*P*< 0.001, [c]*P*< 0.01, [d]*P*< 0.05 [ns]- non significant Methonalic extract of Anogeissus latifolia (100,200 mg/kg) and atrovastatin (10mg/kg) compared with hyperlipidemic control.; [e]*P*< 0.001 Methonalic extract of *Anogeissus latifolia* (200 mg/kg) compared with Methonalic extract of Anogeissus latifolia (100, mg/kg).

Table.6.Effect of Methonalic extract of *Anogeissus latifolia* on kidney antioxidants in cholesterol fed hyperlipidemic animals

Group	Dose (mg/kg)	CAT (µM of H_2O_2 consumed/min/ mg of protein)	SOD (U/mg of protein)	GPx (µg of GSH consumed/min/ mg protein)	GSH (µg of GSH consumed/min/ mg protein)	TBARS (ŋmoles of MDA released/mg protein)
Normal control	Saline (5 ml/kg)	553.81± 40.36	24.35± 1.16	66.10± 2.33	7.939± 0.78	62.31± 5.18
Hyperlipidemic control	Cholesterol fed (25mg/kg)	159.60± 18.04[a]	14.38± 1.32 [a]	23.38±1.56[a]	2.833± 0.48 [a]	351.10± 9.38 [a]
Methonalic extract of *Anogeissus latifolia*	100	350.01±9.255[b]	44.24±0.922[b,d]	53.38±0.806[b]	11.03±0.22[b]	287.73± 1.81[b]
Methonalic extract of *Anogeissus latifolia*	200	669.56±9.92[b,]	52.18± 0.83[b]	79.01±2.56[b]	13.62± 0.37[b,]	156.01±0.81[b]
Atrovastatin	10	299.62± 12.47[b]	45.41± 5.78[c]	60.48± 1.3[b]	6.54±0.14[c]	46.55±0.49[b]

Values are expressed as mean ± S.E.M., (n = 6); [a]*P*< 0.001 hyperlipidemic control compared with normal control ;.[b]*P*< 0.001, [c]*P*< 0.01, , Methonalic extract of Anogeissus latifolia (100,200 mg/kg), and atrovastatin (10 mg/kg) compared with hyperlipidemic control.

Table.7.Effect of Methonalic extract of *Anogeissus latifolia* on lipid profiles in triton induced hyperlipidemic model

Group	Dose (mg/kg)	TC (mg/dl)			TG (mg/dl)			HDL (mg/dl)			LDL (mg/dl)			VLDL (mg/dl)		
		0h	22h	44h	0h	22h	44 h	0h	22h	44h	0h	22h	44h	0h	22h	44h
Normal control	Saline (5ml/kg)	84.12 ±1.2	84.87 ±3.3	84.23 ±4.3	115.2 ±1.2	118.21 ±0.7	118.7 ±1.2	36.76 ±1.2	38.70 ±1.6	41.67 ±2.5	24.23 ±0.2	23.67 ±0.3	18.51 ±2.5	23.04 ±0.4	22.48 ±2.2	23.41 ±0.4
Hyperlipide-mic control	Cholesterol fed (25mg/kg)	86.43 ±2.8	159.12 ±1.1[a]	190.7 ±1.8[a]	114.6 ±1.4	260.82 ±11.3[a]	233.3 ±8.8[a]	35.26 ±3.2	22.40 ±1.2[a]	21.6 ±0.6[a]	24.78 ±0.3	85.21 ±2.1[a]	122.0 ±1.9[a]	22.92 ±0.9	52.42 ±9.5[a]	46.67 ±1.7[a]
Methonalic extract of *Anogeissus latifolia*	100	77.32 ±2.7	165.7 ±4.6[ns]	136.01 ±0.5[c]	110.6 ±1.8	236.2 ±3.9[ns]	155.02 ±4.0[b]	31.76 ±1.9	23.80 ±0.9[ns]	37.5 ±1.0[b]	23.64 ±0.2	94.37 ±1.7[ns]	67.01 ±1.0[c]	22.12 ±0.1	47.63 ±1.7[ns]	30.65 ±1.0[b]
Methonalic extract of *Anogeissus latifolia*	200	75.30 ±2.5	174.01 ±5.8[ns]	116.7 ±0.8[b]	116.7 ±3.4	265.3 ±3.5[ns]	140.3 ±4.3[b]	40.12 ±1.2	22.90 ±1.3[ns]	36.07 ±1.3[b]	19.91 ± 0.1	98.67 ±2.5[ns]	52,12 ±2.0[b]	23.34 ±0.3	53.40 ±5.8[ns]	28.15 ±0.8[b]
Atrovastatin	10	81.56 ±1.7	90.80 ±2.8[b]	136.7 ±8.8[c]	118.1 ±2.8	121.6 ±0.6[b]	185.12 ±5.7[b]	41.16 ±1.5	41.60 ± 1.9[b]	36.8 ±0.8[b]	16.76 ±0.1	25.92 ±2.0[ns]	63.21 ±9.8[c]	23.64 ±0.6	23.25 ±4.7[b]	36.47 ±0.8[c]

Values are expressed as mean ± S.E.M. (n = 6).[a].*P*< 0.01 hyperlipidemic control compared with normal control ;[b]*P*< 0.001, [c]*P*< 0.01, [d]*P*< 0.01 and [ns]-non signifiant Methonalic extract of *Anogeissus latifolia* (100, 200 mg/kg) and atrovastatin (10 mg/kg) compared with hyperlipidemic control.

High cholesterol fed induced hyperlipidemia model: Nutrition plays an important role in the etiology of hyperlipidemia and atherosclerosis. Cholesterol is commonly present in cooking oil and it is essential for formation and maintenance of cell membranes. It helps the cells to resist changes in temperature and protects and insulates nerve fibers, formation of sex hormones progesterone, testosterone, estradiol, cortisol, production of bile salts, digest food, formation of vitamin D in the skin when exposed to sun light. But, high content of cholesterol containing food intake leads to hyperlipidemia and its increases the cardio vascular risks. To prevent hyperlipidemia related diseases, different classes of therapeutic agents are used. There are many models are used to study the antihyperlipidemic activity of drugs and other formulation in animals including high dose of cholesterol feeding animal model also. Cholesterol feeding alone will not effectively elevate the serum TG level. Hence, high dose of cholesterol administrated with high level of saturated fats (example-coconut oil) is helpful to increase serum TG level significant manner in animal models. Therefore, in present study, high dose cholesterol was administered along with coconut oil to the animals to study the lipid lowering effect of Methonalic extract of *Anogeissus latifolia.*

Diet containing saturated fatty acids increases the activity of HMG-CoA reductase, the rate-determining enzyme in cholesterol biosynthesis; this may be due to higher availability of acetyl CoA, which stimulated the cholesterogenesis rate. Moreover, this could be associated with a down regulation in LDL receptors by the cholesterol and saturated fatty acids in the diet, which could lead to elevation of serum LDL levels either by changing hepatic LDLR (LDL-receptor) activity or LDL production rate or both. The activity of cholesterol ester transfer protein (CETP), a key enzyme in reverse cholesterol transport and HDL metabolism increase in high fat diet and mediates the transfer of cholesterol esters from HDL to triglyceride-rich particles in exchange for triglycerides. This leads to increased plasma concentrations of TGs and decreased concentrations of HDL[81] Moreover, in normal condition, LCAT enzyme is involved in the transesterification of cholesterol, maturation of HDL and flux of cholesterol from cell membranes into HDL. This action of enzyme tends to decrease in diet-induced hypercholesterolemia. In this study, elevated level of serum triglycerides, cholesterol, LDL and VLDL accompanied by decrease of serum HDL was observed as compared to normal control rats after the administration of high dose cholesterol to the rats.

The oral administration of Methonalic extract of *Anogeissus latifolia* (100 and 200 mg/kg) and reference standard atrovastatin (10 mg/kg), a HMG-CoA inhibitor, showed a significant decrease of serum triglycerides, cholesterol, LDL and VLDL and significant increase of serum HDL levels. Administration of Methonalic extract of *Anogeissus latifolia* (200 mg/kg) showed better results when compared to Methonalic extract of *Anogeissus latifolia* (100 mg/kg).

The MEALL treatment significantly increased the protective HDL level and decreased the atherogenic LDL and VLDL levels. This is may be due enhancement of LACT enzyme activity by Methonalic extract of *Anogeissus latifolia*, which may mobilize cholesterol and triglycerides from peripheral cells or serum to liver that leads to increases the level of HDL. This pathway termed 'reverse cholesterol transport' where it is catabolised and excreted out of the body.

Phospholipids are vital components of biomembrane and these are important for integrity micro viscosity and survival of cells. In hyperlipidemic condition, increased level of plasma phospholipids observed which may be due to membrane damage caused by increased lipid peroxidation. Increased lipid peroxidation thought to be a consequence of oxidative stress. Oxidative stress enhances the generation of free radicals and it may damage cell membrane. In hyperlipidemia, increased lipid peroxidation occur which alter the enzymes, involved in peroxide metabolism, that leads changes in LDL receptors conformation possible resulting in an exposure of fatty acid to oxygen free radical that enhance the faster rate of lipid peroxidation. Oxidized LDL thought to promote atherogenesis by increased level of thiobarbituric acid reactive substance (TBARS). In present study, increased level of TBARS was observed in high dose cholesterol treated rats compared to normal control rats which may be due to lipid peroxidation. After the treatment with both the doses of MEALL and MEALL significantly decrease the TBARS levels compared to hyperlipidemic control animals. Also, the enhancement of antioxidant enzyme activity (CAT, SOD, GSH and GPx) was observed after the treatment of MEALL and MEAL in hyperlipidemic rats and this action may be due to inhibits the free radical generation due presence of flavonoids and phenolic compounds.

Hyperlipidemia induced metabolic alteration and oxidative stresses are causing certain degree of hepato-cellular damage and renal toxicity. In present study, observed elevated serum markers of hepatic (SGOT, SGPT, ALP,) and renal (creatinine) functions is suggestive of hyperlipidemia induced hepatic and renal damage. The MEALL treatment significantly reduced the increased SGOT, SGPT, ALP and creatinine levels in hyperlipidemic control animals compared normal control. These actions possibly due to protective nature of Methonalic extract of *Anogeissus*

latifolia on hepatocellular damage and renal toxicity caused by hyperlipidema.

Triton Model: Triton induced hyperlipidemia in rats is an acute model for the primary screening of drugs having lipid lowering action. TR acts as a surfactant and suppresses the lipases action by blocking the uptake of lipoproteins from circulation from extra hepatic tissues. TR induced hyperlipidemia occurs in 2 phases. Phase 1 (synthesis phase), it is thought to be due to increased hepatic synthesis of cholesterol, which reaches the elevated lipid level at the end of 24 hours though the ability to interfere with the uptake of plasma lipid levels, by the tissues. Drugs interfering with cholesterol biosynthesis were shown to be active in this phase. In phase 2 (excretory phase), the elevated lipid levels almost reach normal by the end of 48 h while drugs interfering with cholesterol excretion and metabolism were shown to be active in this phase. The biphasic nature of TR induced hyperlipidemia is helpful in understanding the mode of action of hypolipidemic agents. The drugs possessing antihyperlipidemic action act on either phases or any one phase. The drugs act on phase 1 by interfering with cholesterol biosynthesis thereby decreasing blood lipid profiles levels. Also, many drugs act on phase 2 by interfering with cholesterol excretion and metabolism. In our study, administration of MEALL to the triton induced hyperlipidemic animals did not reduced significantly lipid profile levels at 22 hours, i.e. in phase I, but the significant reduction of lipid profile levels were observed at 44 h i.e. in phase 2. Therefore, Methonalic extract of *Anogeissus latifolia* may be act via excretory phase to produce their hypolipidemic activity. Also, administration of METGL and METGB showed significant increase in HDL level. This action may be due to LCAT and inhibition of the action of TG-lipase on HDL, which may contribute for a rapid catabolism of blood lipids though extra hepatic tissues.

4. CONCLUSION

The phytochemical constituents analysis of Methonalic extract of *Anogeissus latifolia* showed the presence of alkaloids, carbohydrates, flavonoids, phenolic compounds, glycosides, proteins and aminoacids, saponins, steroids and sterols. The total phenolic content in Methonalic extract of *Anogeissus latifolia* were found to be 318.7 µg/mg of extract respectively equivalent to gallic acid. The total flavonoids component in Methonalic extract of *Anogeissus latifolia* was found to be 343.75 µg/mg of extract and of extract equivalent to quercetin. Methonalic extract of *Anogeissus latifolia* was significantly reduced total cholesterol, triglycerides, LDL and VLDL levels as well as increased HDL level in high fed cholesterol and Triton X 100 induced hyperlipidemic models. Also, the atherogenic index of Methonalic extract of *Anogeissus latifolia* treated groups showed was significant reduction compared to hyperlipidemic control. In Methonalic extract of *Anogeissus latifolia* treated hyperlipidemic animals, the antioxidant status was restored towards normal levels which indicated that both extracts has potential to reduce the lipid peroxidation and increases the antioxidant levels. Based on above models, the possible lipid powering mechanism of Methonalic extract of *Anogeissus latifolia* may be due to increasing the cholesterol and triglyceride excretion by enhanced activity of LCAT enzyme. Therefore, we conclude that present studies support lipid lowering action of methanol extract of *Anogessius latifolia* leaves in animal models. Moreover, the results clearly revealed that methanol extract of *Anogessius latifolia* (200mg/kg) possesses higher lipid lowering action than (100mg/kg) Hence, *Anogessius latifolia* leaves have potential to reduce the atherosclerosis which will directly help to reduce the cardiovascular risks associated with hyperlipidemia related cardiovascular disorders.

REFERENCES

1. George M, Craig W, Text book of pharmacology second edition, Kundli, Sanat Printers, Kundli, 2008.

2. Smith DG, Epidermiology of dislipidemia and economic burden on the health care system, American Journal of Managing Care, 13 (3), 2007, 68-71.

3. Toth PP, subclinical atherosclerosis: what it is, what it means and what we can do about it, International Journal of Clinical Practice, 62(8), 2008, 1246-56.

4. Goldenburg N, Glueck C, Efficacy, effectiveness and real life goal attainment of statins in managing cardiovascular risk, Vascular Health and Risk Management, 5(1), 2009, 369-76.

5. Eddouks A, Lemhadri, Michel JB, Hypolipidemic activity of aqueous extract of *Capparis spinosa* L,in normal and diabetic rats, Journal of Ethnopharmacology, 98(5), 2005, 345-350.

6. Nessa A, Uddin M, Ferdousi S, Hussain MA, Diet and life sytle Modification be able to reduce risk of coronary heart diseases, J of Bangladesh Soc of Physiol, 4(1), 2006, 40-41.

7. David E Golan, Armen HTashjian jr, Text book of principles of pharmacology second edition, New Delhi, Wolter Kluwer (India) Pvt.Ltd., 2008.

8. Chan DC, Barrett PH, Watts GF, Lipoprotein transport in the metabolic syndrome: Pathophysiological and Therapeutic Lessons from Stable Isotope Studies. Clin Sci (Lond), 25, 2004, 31-48.

9. Daniels TF, Killinger KM, Michal JJ, Wright RW Jr, Jiang Z, Lipoproteins, cholesterol homeostasis and cardiac health, Int J Biol, 5(5), 2009, 474-88.

10. Ikonen E, Mechanisms for Cellular Cholesterol Transport: Defects and Human Disease. Institute of Biomedicine/Anatomy, Physiology Reviews, 86(4), 2006, 1237-61.

11. Rang HP, Dale MM, Text book of pharmacology.6th ed. Philadelphia; Churchill living stone, 2010, 324-334.

12. Sharma HL, Sharma KL, Text book of principals of pharmacology, Hyderabad, Paras publisher, 2010, 415-425.