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EFFECTS OF *ANETHUM GRAVEOLENSE* LINN. EXTRACT ON BILE DUCT LIGATION INDUCED HEPATIC FIBROSIS IN RATS

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Keywords:

Anethum graveolence L., Bile duct ligation, Flavonoids, Cholestasis, Oxidative injury, TGF-β1

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ABSTRACT: The present study investigated the protective role of methanolic extract from *Anethum graveolense* L. (AGME) on bile duct ligation (BDL) produce hepatic fibrosis in the rat. BDL rats were divided into four groups, which received orally distilled water or AGME (200 and 400 mg/kg) for continuously 28 days. The BDL induced hepatic fibrosis by anti-fibrotic effect of AGME in the rats determined by serum level of alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBL), direct bilirubin (DBL), alkaline phosphatase (ALP), creatinine (CRT), glucose (GLU), triglycerides (TGL), cholesterol (CHOL), total protein (TP), albumin (ALB), transforming growth factor beta-1 (TGF-β1) and oxidative parameter like glutathione (GSH), malondialdehyde (MDA), total superoxide dismutase (SOD) and nitric oxide (NO) level. Biochemical estimation was complemented by histopathological measurement of the liver. Phytochemical in AGME were determined by qualitative and high-performance liquid chromatography (HPLC) analysis. All the serological level was elevated on treated with BDL group alone than in the sham control group ($P<0.01$). Treatment with AGME these elevations was mostly diminished. Also, increase in NO level, and AGME doses produced hepatic MDA in BDL induced cholestasis. Furthermore, the treatment with AGME significantly diminished the serum level of TGF-β1, fibrogenic cytokine. Histopathological finding further showed the protective effect of AGME by BDL induced liver fibrosis in rats. Phytochemical analysis of AGME shown that it contained myricetin, rutin, vitexin, hyperoside, and kaempferol was determined by AGME. These beneficial roles of AGME might be an effective antifibrotic drug in cholestatic liver disease.

INTRODUCTION: Failure of bile duct leads to decrease the bile secretion and also the storage of toxic substance within the liver. It is evident from leukocyte infiltration, portal tract expansion, the proliferation of liver cirrhosis and fibrosis [1, 2]. The mechanism involved in the development of cholestatic liver, oxidative stress may be the main causative factor. This cholestatic showed by activation of the collagen gene transcript [3, 4].

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The antioxidant avoids the activation of the hepatic stellate cell through type 1 collagen in liver fibrogenesis. The defense mechanism of the endogenous antioxidant could play the main role in blocking major damage in cholestatic liver [5]. The deposition of extracellular matrix protein is the main pathological fact of hepatic fibrosis.

It is well documented, that the multifunctional cytokine TGF-β plays a vital aspect in the development of liver cirrhosis and fibrosis by its trigger effect on the matrix protein generation and suppression effect on the matrix protein removal [6]. TGF-β is large super-family and prototypic member of the secreted protein that has been used in many therapeutic aspects to minimize hepatic fibrosis.

Moreover, clinically effective treatment for therapeutic aspect and liver fibrosis against the progression of liver fibrosis is desired [7, 8, 9]. Silymarin, a hepatoprotective with activity against anti-hepatitis virus (HCV), diminished the expression of TGF- and inhibited the hematopoietic stem cell (HSC) by platelet-derived growth factor and for cirrhosis activation of BDL project [10]. Herbal product and therapies those derived from the natural compound such as silymarin may well use in the care of the patient with HCV, but adequately not yet designed for the clinical trial [11]. Recently, the cure of liver disease has been still a competition for modern drug treatment for liver function or regeneration of new hepatic cells. The immuno-suppressants and corticosteroids are found in the current market but have the major side effect [12]. Silymarin is the only molecule that available no other. It is necessary to discover for new molecules [13]. From ancient time the fruit of *Anethum graveolens* L. common name Dill belong to the family Umbelliferae (Authority: Linnaeus) is used as the spice and the whole aerial part of the plant is used as vegetable particularly in north India. The height of the plant is approximately 150 cm long, round stem and 2-5 branches arise from the base of the stem. The color of the flower is yellow. The seed color after-ripening is a light brown color and produce an aromatic odor [14].

Anethum graveolens L. contains mainly con are flavonoids, phenolic compounds and essential oil [15]. The *Anethum graveolens* L. shows significant anti-stress, antioxidant [16], antibacterial [17] and cardioprotective agent [18]. Although, the AGME showed the beneficial effect of the acute liver failure had been broadly studied using broadly hepatic injury made induced by chemical, little is recognized as its ability to safe hepatic fibrosis to the BDL induced cholestasis. The common pathological condition in cholestasis that can be reproduced by surgical ligation in rodent of the common bile duct. The present study was the investigation of the protective role of the standardized AGME on hepatic fibrosis and oxidative stress in rat with BDL.

MATERIALS AND METHODS:

Drug and Chemicals: Assay kit for GSH, thiobarbituric acid reactive substances (TBARS) as well as NO were ordered from Span Diagnostics Ltd., Surat, India. The assay kit of SOD was bought from Sigma-Aldrich [P] Ltd. Delhi. Standard drug Sylibon- 140 (Silymarin) purchased from the market manufactured by Microlab Limited Solon, Himachal Pradesh, India. The biochemical estimated kit for ALT, AST, ALP, CHOL, TGL, GLU, ALB, CRT, TBL, DBL and TP from Transasia biochemical Limited Solon, Himachal Pradesh, India. TGF-β1 ELISA kit was available from Sigma-Aldrich [P] Ltd. Delhi. The other reagents taken in the experiment were of analytical grade.

Collection and Authentification of Plant: The plant *Anethum graveolens* L. was collected from the field of Amethi, Uttar Pradesh, India in February. The authentication of a plant by Dr. R. R. Singh ex-head of the Botany Department, Lucknow University, Lucknow, India, authenticated and specimen voucher reference number is LU-DP-288 has been deposited in herbarium departmental.

Preparation of Plant Extract: The aerial part of *Anethum graveolens* L. (leaves, stem, and flower) material was air dried at room temperature for 15 days and after it was made to the uniform powder. 2 kg powder plants were extracted with 6 L methanol at 25 °C in a Soxhlet extraction for 2 days. After extraction, the methanol solution was dried at room temperature, and it was suspended in distilled water and fractionation with normal hexane, diethyl ether, chloroform, ethyl acetate, and methanol. All extract and fraction were allowed to preliminary pharmacological study by using bile duct ligation-induced model and found that the methanol fraction showed with potential activity, therefore, methanol fraction was selected for detailed study. The methanol fraction, of plant and kept at temperature 4 °C *in-vivo* experiment.

HPLC of AGME: 50 mg of powder plant was extracted with 6 ml of 25% hydrochloric acid and 20 ml methanol for 60 min. The extract was filtered into a volumetric flask than residue was heated twice with 20 ml of methanol for 20 min to get the extract. The combined extract was poured with methanol to 100 ml. 5 ml portions of the solution were filtered and transferred to a volumetric flask and diluted with 10 ml of methanol. 8 µl samples were injected into the HPLC apparatus [19]. After these were analyzed on Shimadzu HPLC system

(LC2010C). Separation was passed out through column ZORBAX ECLIPSE, XDB-C8, (5 µm; 4.6 ×150 mm) with Spectra-Focus detector of UV-VIS, injector-auto sampler. Solvent A and B (0.05% trifluoroacetic acid and 0.038% trifluoroacetic acid in 83% methanol (v/v) with the following gradient: 0-5 min, 15% B in A, 5-10 min, 50% B in A, 10-15 min, 70% B in A. The measurement of flow rate was 0.8 ml/min, and injection volume was 8 µl. Five standard compounds including myricetin, rutin, vitexin, hyperoside, and kaempferol were run for comparative detection and were optimized than start the calibration curves were focused for every compound in the between of sample quantity 0.02-0.5 µg. Each quantitative data were evaluated using the analytic software.

Acute Toxicity Study: Acute toxicity study was carried out for the fractions, AGME according to the Organization for Economic Co-operation and Development (OECD) 420 guidelines (OECD, 2001). Wister albino rats were divided into groups comprising six animals in each group. The fractions were administered to different groups in doses ranging from 100-2000 mg/kg b.w.p.o. They were observed for signs of toxicity and mortality for 72h.

Animal and Treatment: The Wister albino rats of any sex, weight about 150 to 200 g were used in this study. All animal were kept in standard cages at the room temperature (25 ± 3 °C) with a 12-h dark-light cycle, water *ad libitum* and fed with standard diet and standard animal care were followed. A total number of rats is 30 for five groups. The fasting time of the animal is 16 h and provided water for the experiment.

All the experiments were done in the pharmacology department, RRS College of Pharmacy, Amethi and the approved by the Institutional Animal Ethical Committee (IAEC/CPCSEA/SBCP1045/Ere/07). The animals were acclimatized before conducting the experiments. The animals were housed in cages of 40×20×15 cm made up of polypropylene. The performance of the experimental work was started after getting approval from the Institutional animal ethics committee. All experimental work was conducted in Department of Pharmacology, Rajarshi Rananjay Singh College of Pharmacy, Amethi - 227405, Uttar Pradesh, India. The animals were anesthetized through pentobarbitone (45 mg/kg, i.p) and exposed their common bile duct between the abdominal incision using a double tie with silk thread was ligated to the common bile duct [20]. The first tie was made below the point of hepatic duct and the second tie was made above passage of the pancreatic after incision of BDL rats were separated into four groups (n=6) which orally treatment distilled water or AGME (200 and 400 mg/kg/day) for 28 days sequence day. AGME was diffused in distilled water. The sham control group (n=6) was surgery by making for incision on abdominal. Without any use of ligation and given up to 28 days in distilled water orally **Table 1**.

TABLE 1: DESIGN OF EXPERIMENTAL GROUP FOR THE HEPATOPROTECTIVE

Group A	Sham Control group
Group B	BDL alone group
Group C	BDL + Silymarin (Provided Silymarin 50 mg/kg)
Group D	BDL + AGME- 200 mg/kg (Provided AGME 200 mg/kg)
Group E	BDL + AGME- 400 mg/kg (Provided AGME 400 mg/kg)

BDL: Bile duct ligation; AGME: Methanolic extract from *Anethum graveolence* L.

After 28 days, the BDL operation, the animal's sacrificed by cervical dislocation and the collection of blood, the blood sample was allowed to keep for 30 min at room temperature after centrifuged at the 3000 rpm for 15 min. To separate the serum. All the serum was analyzed intended for various serological parameters *i.e.* ALP, AST, ALB, ALT, TBL, CLU, CRT, TGL, TP, DBL, and CHOL. The weight of the liver was taken and prothrombin time was recorded. The livers were carefully removed after the animals were dissected and washed with the 0.9% of the saline solution. The liver sample was kept in formalin solution of 10% formaldehyde for biochemical and histopathological studies.

Serum Biochemical Evaluation: At the end of the therapy period, all animals fasted to allow for 12 h, after this blood sample was taken by cardiac puncture using sterile disposable syringes by cervical dislocation. Sera were partitioned out by centrifugation at 3000 g for 15 min and kept at -20 ºC until analysis. Then, animals were sacrificed for the collection of the liver. The damaged liver was assessed by the estimation of serum activities of the ALT, ALP, AST, TGL, CHOL, GLU, CRT, ALB, TBL, DBL and TP using commercially available test kits [21, 22].

Estimation of Antioxidant Activity: The MDA content, the estimated to lead to a lifeless product of lipid peroxidation, was measured the use of the reduction method of thiobarbituric acid available kit Sigma-Aldrich [P] Ltd. Delhi.

Hepatic GSH levels were calculated by using the improved 5, 50-dithiobis-(2-nitrobenzoic acid) method Sigma-Aldrich [P] Ltd. Delhi. The total SOD activity in liver homogenates was estimated through the superoxide dissolution detected using a tetrazolium salt from Span Diagnostics Ltd., Surat, India [23].

Estimation of Nitric Oxide/Nitrite Level: The nitric oxide was measured according to the method of Vishwakarma *et al*. (2017) [24].

Estimation of Plasma TGF-*β*1 Levels: Plasma TGF-*β*1 level was estimated using the ELISA kit Sigma Aldrich [P] Ltd., Delhi, according to the manufacturer's guidelines [6, 25].

Histopathological Studies: Studies of histopathology were carried out to locate the degree of harm through staining the part of the isolated liver organ and analyzing by using a microscope. The liver used to be separated from the sacrificed animals and then cleaned with regular saline (0.9%). After the washing, livers have been cut into 2-3 portions (approx. 6 cm) and stored in phosphate buffer with 10% formaldehyde solution.

The liver was cut into the thickness of 5 mm after embedding in paraffin wax and amp; stained with hematoxylin-eosin stain. Collected four random samples of every liver tissue pattern were stained with eosin and hematoxylin. The degree of fibrosis used to depend which mentions with portal inflammation, confluent necrosis, focal necrosis, focal inflammation, piecemeal necrosis, apoptosis, and fibrosis. Each section was also noted for the number of biliary canals in the site of 5 portal [5].

Statistical Analysis: The data were analyzed as mean ± SD. The result using one way of ANOVA followed *via* Dennett's test for a significant an interrelation between many groups using graph pad computer software, version 5.01 and the country of the graph pad prism program by the United States. The $P<0.01$ was considered to the significant from BDL group.

RESULTS: The period of 28 days in the experimental group, there was not any mortality. However, all BDL group rats showed major hepatomegaly, jaundice, slight splenomegaly and ascites except for sham control rats. To explore the effects of BDL in the liver, *Anethum graveolence* L. Silymarin, on liver functions we were evaluated serological parameter tests for ALT, AST and more in blood samples. We determine the oxidative levels in the liver and finally. Histopathology of the liver was used to determine the tissue damage.

Chemical Composition of AGME:

Qualitative Analysis of AGME: Phytochemical analysis of AGME demonstrated the presence of flavonoids, reducing sugar, saponins, steroid, tannin. The acute toxicity study, no toxic symptoms or death was seen during the period of the limit test.

HPLC Quantification of Flavonoids: Preliminary HPLC studies revealed the well-resolved peak of MELA. The probed compounds in the AGME were express by integration of the peak areas of the 220nm using an external calibration method for each analyte. The main active flavonoids in the extract were myricetin, rutin, vitexin, hyperoside, and kaempferol.

Acute Toxicity Study: This study caused that the LD_{50} was more than 2000 mg/kg as none of the rats showed harmful symptoms after the dose of AGME for two weeks.

Serological Test for Liver: As observed, in Group C the animal surgery for BDL. BDL showed significantly raised the level of the hepatic enzyme. AST, ALT, ALP, DBL, TBL, RBS, SRL, HDL, STL and decreased the levels of TP, ALB, compared to Group A. The decrease and increase in case of TP, ALB significantly in the Group D and E that received BDL + AGME- 200 mg/kg, BDL + AGME- 400 mg/kg of doses 200 and 400 mg/kg after BDL group, respectively that compared with Group B that treated with BDL. In the Group E, BDL + AGME- 200 mg/kg at the dose of 400 mg/kg diminished the hepatic enzyme to normal level (with the Group A no significant difference), and was as a potent as silymarin at 50 mg/kg in Group B that prevents the effect of BDL **Table 2** and **3**.

TABLE 2: EFFECT OF AGME ON SERUM BIOCHEMICAL PARAMETERS IN BDL INDUCED CHOLESTASIS IN RATS

Group	DOSE (mg/kg)	AST (U/L)	ALT (U/L)	ALP (U/L)	TBL (mg/dl)	DBL (mg/dl)
Sham Control	-	10.16±0.75	28.50±2.58	86.33±1.86	0.35±0.07	0.11±0.01
BDL alone	-	54.16±2.32[a]	123.83±1.32[a]	156.33±2.25[a]	4.42±0.96[a]	0.83±0.08[a]
BDL+Silymarin- 50 mg/kg	50	18.33±1.03[b]	36.50±1.17[b]	105.00±3.16[b]	0.75±0.05[b]	0.22±0.03[b]
BDL+AGME- 200 mg/kg	200	33.83±7.11[b,c]	39.33±0.82[b,c]	128.33±19.09[b,c]	0.96±0.06[b,c]	0.26±0.04[b,c]
BDL+AGME- 400 mg/kg	400	21.83±2.48[b,c]	38.83±0.98[b,c]	123.33±12.82[b,c]	0.93±0.08[b,c]	0.24±0.04[b,c]

Data expressed as mean ± SD (In survival animal) After BDL treatment, the rats in AGME treated group was orally given AGME (200 and 400 mg/kg) one time a day for 4 weeks. Sham control group were orally treatment the same amount of saline water as the BDL treated animals that provide AGME. n=6, P-value BDL *vs.* vehicle<0.01; P value BDL *vs.* treatments <0.01. AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatise; TBL: Total bilirubin; DBL: Direct bilirubin; BDL: Bile duct ligation; AGME: Methanolic extract from *Anethum graveolense* L.

TABLE 3: EFFECT OF AGME ON SEROLOGICAL PARAMETERS IN BDL INDUCED CHOLESTASIS IN RATS

Group	DOSE (mg/kg)	CRT (mg/dl)	GLU (mg/dl)	TGL (mg/dl)	CHOL (mg/dl)	TP (g/dl)	ALB (g/dl)
Sham Control	-	0.81±0.13	102.50±13.32	73.33±2.73	35.24±3.22	7.88±0.26	4.71±0.20
BDL alone	-	2.49±.43[a]	192.50±9.35[a]	200.30±7.07[a]	90.42±7.07[a]	4.78±0.18[a]	2.37± 0.28[a]
BDL+Silymarin-50 mg/kg	50	1.2±0.12[b]	123.17±4.26[b]	110.40±4.47[b]	46.74±2.7[b]	7.4±0.13[b]	4.23±0.22[b]
BDL+AGME- 200 mg/kg	200	1.39±0.07[b,c]	135.03± 8.9[b,c]	128.83± 8.61[b,c]	52.00±5.06[b,c]	7.17±0.23[b,c]	3.89±0.23[b,c]
BDL+AGME- 400 mg/kg	400	1.32±0.1[b,c]	143.67±3.83[b,c]	136.50±7.96[b,c]	56.33±5.88[b,c]	6.72±0.53[b,c]	3.50±0.22[b,c]

Data expressed as mean ± SD (In survival animal) After BDL treatment, the rats in AGME treated group was orally given AGME (200 and 400 mg/kg) one time a day for 28 days. Sham control group were orally treatment the same amount of saline water as the BDL treated animals that provide AGME. n=6, P value BDL *vs.* vehicle <0.01; P value BDL *vs.* treatments <0.01. CRT: Creatinine; GLU: Glucose; TGL: triglycerides; CHOL: Cholesterol; TP: Total protein; ALB: Albumin; BDL: Bile duct ligation; AGME: Methanolic extract from *Anethum graveolence* L.

Antioxidant Parameter of Liver: Lipid peroxidation product, SOD and GSH are a marker of oxidative damage, which was measured in liver homogenates.

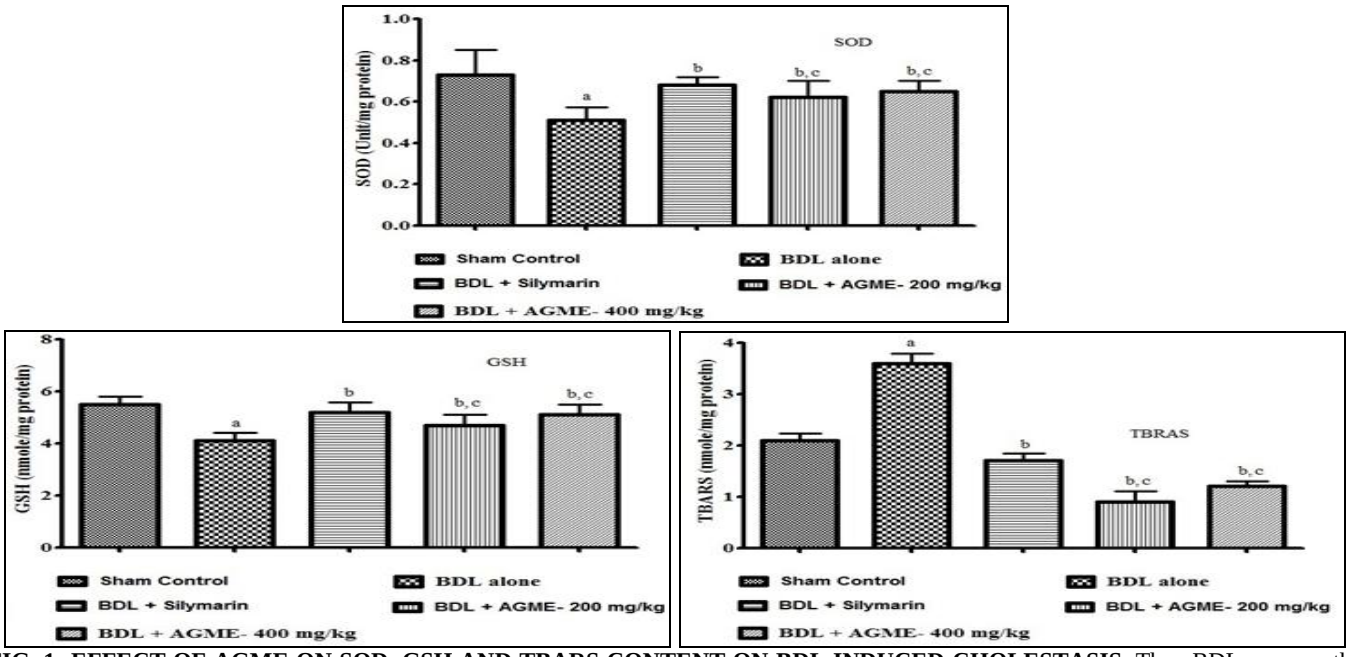


FIG. 1: EFFECT OF AGME ON SOD, GSH AND TBARS CONTENT ON BDL INDUCED CHOLESTASIS. Than BDL process, the rats in AGME treated groups were orally treatment AGME at 200 and 400 mg/kg as per body weight per day for 28 days. The Sham control groups were orally treated with the same amount of saline as the BDL operation animals that received AGME. All values were expressed as mean + SD. [a]P<0.01, show significant change when compared with the sham control group. [b]P<0.01, show significant change when compared with the operation with BDL alone group. The level of SOD and GSH has decreased in BDL alone but increased in TBARS, but when given doses of AGME it significantly increased in SOD and GSH but decrease in TBARS. BDL: Bile duct ligation; AGME: Methanolic extract from *Anethum graveolense* L; SOD: Superoxide dismutase; GSH: Glutathione; TBARS: Thiobarbituric acid reactive substances.

The level of liver SOD and GSH was significantly reduced, together with increase TBARS in the BDL alone group (Group B) compared to the control (P<0.01), Group D and E treated with BDL + AGME -200 mg/kg, BDL + AGME- 400 mg/kg doses of 200 and 400 mg/kg respectively, the

concentration of SOD and GSH increase and decrease concentration of TBARS the towards the normal at dose-dependent manner (P<0.01) for Group D and E compared to the Group B (BDL alone group) **Fig. 1**.

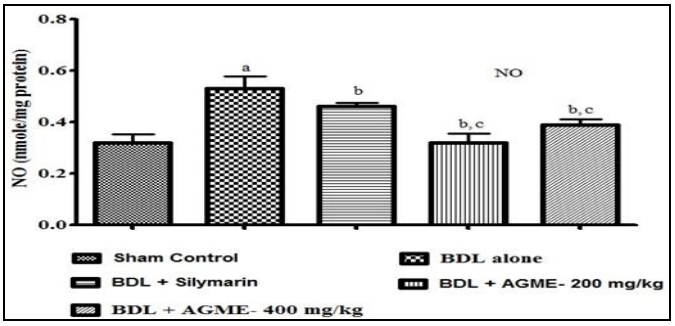
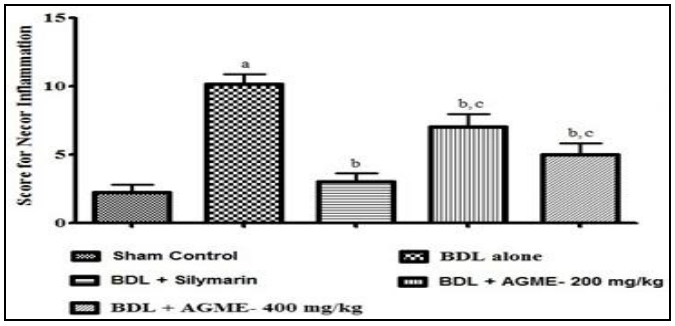
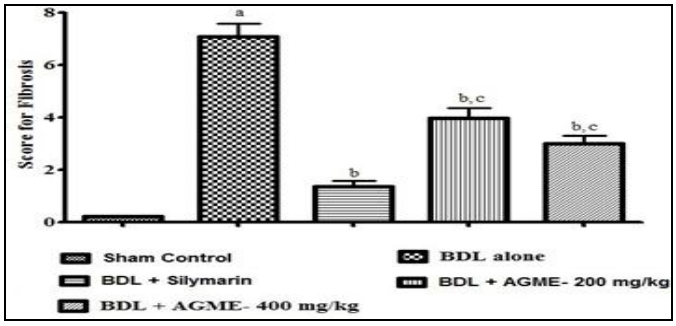


FIG. 2: EFFECT OF AGME ON NO CONTENT IN BDL INDUCED CHOLESTASIS. Than BDL process, the rats in AGME treated groups were orally treatment AGME at 200 and 400 mg/kg as per body weight per day for 28 days. The Sham control groups were orally treatment with the same amount of saline as the BDL operation animals that received AGME. All values were expressed as mean + SD. [a]P< 0.01, show significant change when compared with sham control group. [b]P< 0.01, show significant change when compared with operation with BDL alone group. The level of NO is increased in BDL alone, but when given doses of AGME it significantly decrease. BDL: Bile duct ligation; AGME: Methanolic extract from *Anethum graveolense* L; NO: Nitric oxide.





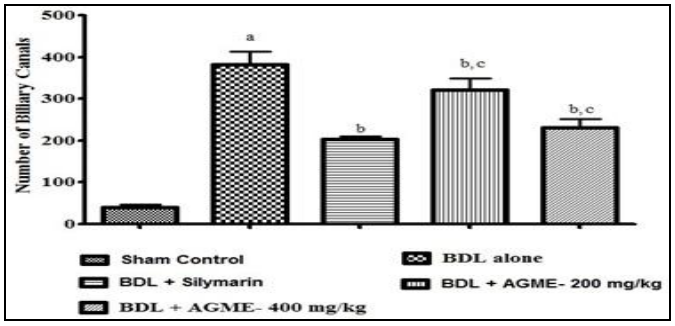


FIG. 3: AFTER BDL PROCESS, THE RATS IN AGME TREATED GROUPS WAS ORALLY TREATMENT AGME AT 200 AND 400 mg/kg AS PER BODY WEIGHT PER DAY FOR 28 DAYS. The Sham control groups were orally treated with the same amount of saline as BDL operation animals that received AGME. All values were expressed as mean + SD. [a]P < 0.01, show significant change when compared with sham control group. [b]P < 0.01, show significant change when compared with operation with BDL alone group. The level of score for necor inflammation, score of fibrosis and number of biliary canals is increased in BDL alone, but when given doses of AGME it significantly decreases the level of all. BDL: Bile duct ligation; AGME: Methanolic extract from *Anethum graveolense* L.

Nitrite Level of Liver: Analysis of NO showed significantly increased levels of the NO on treated with BDL alone group (Group B). The levels of hepatic NO decreased by AGME treatment (P<0.01) **Fig. 2**.

Score of Necor Inflammation and Fibrosis: Score of necor inflammation and fibrosis of the experimental group is summarized in **Fig. 3**. In the sham control group, the liver of animals had a normal hepatic cell, a prominent nucleus with preserved cytoplasm and visible central veins. In BDL operated rats showed that many histologically changes, like the formation of bridging fibrosis in the portal region with the prominent ductular proliferation, lymphocyte infiltration and polymorphonuclear leukocyte and edema in the periportal area. Treatment with AGME (200 and 400 mg/kg) of the BDL rats had markedly diminished histological collagen change, edema, ductular proliferation as well as inflammation occurs on treated with the BDL alone **Fig. 4**.

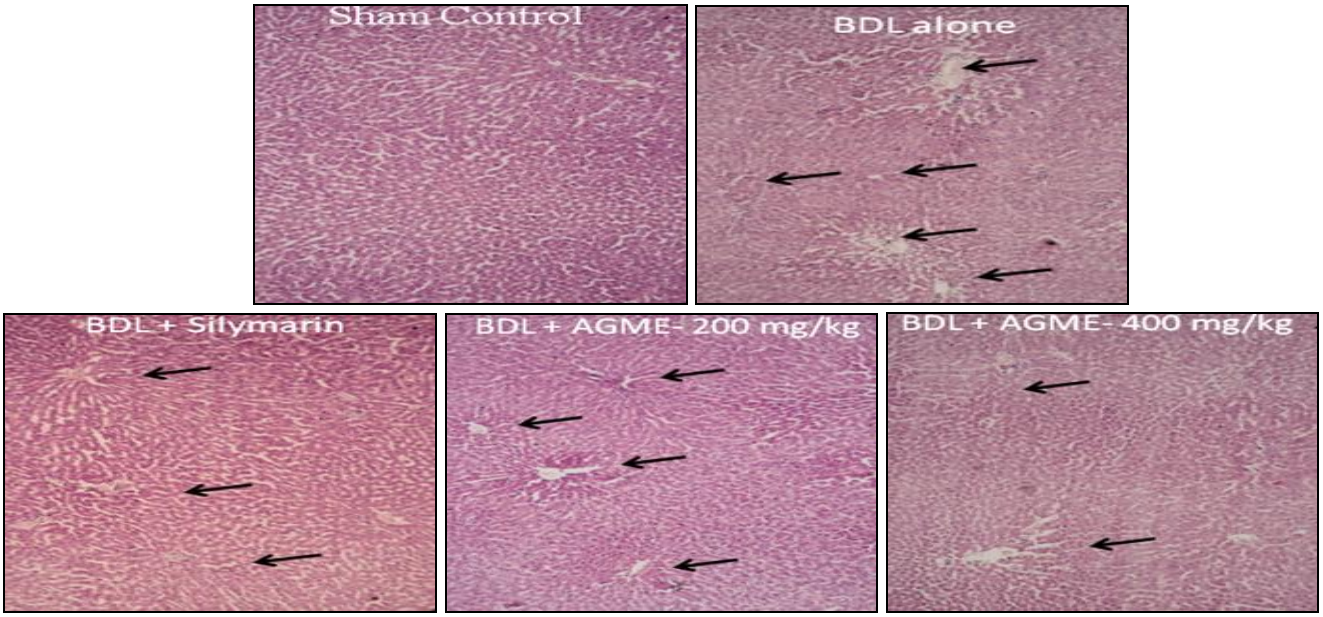


FIG. 4: EFFECT OF AGME ON HISTOPATHOLOGICAL MAKE DIFFERENT IN LIVER OF RATS IN BDL INDUCED CHOLESTASIS. After the BDL operation, the dose of AGME was orally treated at 200 and 400 mg/kg as per body weight per day for 28 days. The sham control group were orally treated the same amount of saline as BDL alone group that received. BDL group showed perivenular inflammatory infiltration and hepatocytic fatty change, diffuse mild hepatocellular vacuolation. The dose of AGME showed perilobular hepatocellular fatty change, mild fatty change, peripheral lobule, less disarrangement and degeneration of hepatocytes, indicating marked hepatoprotection. BDL: Bile duct ligation; AGME: Methanolic extract from *Anethum graveolence* L.

Estimation of Plasma TGF-*β*1 Level: TGF-*β*1, plasma fibrogenic cytokines, which show major role of hepatic stellate cell activation, in the group treated the BDL alone **Fig. 5**. Production of TGF-*β*1 was diminished in AGME treated BDL than treated BDL alone rats.

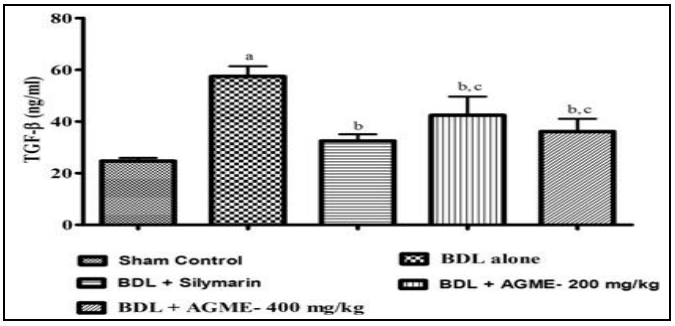


FIG. 5: TGF-B1 OF SERUM LEVEL IN BDL INDUCED CHOLESTASIS. Than BDL process, the rats in AGME treated groups were orally treatment AGME at 200 and 400 mg/kg as per body weight/day for 28 days. The sham control groups were orally treatment with the same amount of saline as the BDL operation animals that received AGME. All values were expressed as mean + SD. $^{a}P<0.01$, show significant change when compared with sham control group. $^{b}P<0.01$, show significant change when compared with the operation with BDL alone group. The level of TGF-β increased in BDL alone, but when given doses of AGME it significantly decrease the level of TGF-*β*. BDL: Bile duct ligation; AGME: Methanolic extract from *Anethum graveolense* L; TGF-β1: Transforming growth factor beta I

DISCUSSION: In the research one of the important animal models on the cholestasis in the complete ligation of the simple BDL model [26]. Obstruction of bile duct and subsequent cholestasis that the concentration of bile acid is increased and toxins within the liver shown inactivation of kuffer cells and hepatic stellate [27]. Serological level of liver is classical marker of blocking cholestasis in clinical observation. Clinically the ratio of AST to ALT was substantially increased in patients with recent fibrosis [28]. In this experiment elevated serum level of the various parameters, together with the ratio of AST/ALT was shown treated with BDL, but the elevations of these parameters were ameliorated in AGME treated BDL group. It is pointed out that BDL induces kind of liver fibrosis that pathogenically and physiologically [29]. The degeneration and death of hepatocellular showed in cholestasis that related to the accumulation of toxic bile salt, which stimulation of oxidative stress through the opening of nicotinamide adenine dinucleotide phosphate oxidase isoform into the pathway of immune activation [30]. It has been implicated that hepatocyte necrosis and apoptosis involved with stimulation of kuffar cells which release tumor necrosis factor and TGF-β [8]. TGF-β is the unregulated in hepatic fibrosis, cause

proliferation in hepatic satellite and generation of the collagen [9]. The expression of increased TGF-β had been published bile duct obstructed hepatic tissue and if induced to pathological retention if extracellular matrix protein [31]. The oxidative stress in the intracellular region is the main contributor to fibrogenesis, and current investigation have expressed the effect of the peroxide radical by induction of the profibrogenic TGF-*β*1, by giving a rational use of intracellular antioxidant as accompanied antifibrotic agent [9]. Many promising drugs obtained from the plant may be protective in a combination of antifibrotic therapy. *Silybum marianum* was exhibited to reduce liver collagen retention in rat biliary fibrosis after to bile duct blockage and baicalein form sho saiko to also mentioned stimulating hepatic satellite cell of antifibrotic properties *in-vitro* [2]. AGME improved liver toxic chemical-induced liver injury, which downward the production of more reactive oxygen species and strongly downward HCV genotype 1*β* replicon of the replication in huh 7 cells [32].

As per data, AGME in a current study reduced BDL induced liver cell GSH depletion inhibited lipid peroxidation and maintained hepatic Cu/Zn SOD function in the liver. Increased the level of TGFβ1 was treated with BDL group treatment with AGME. Persistently, may histopathological aspect of cholestasis like cholestasis. The formation of periportal fibrosis, unrestricted bile duct proliferation, focal necrosis, piecemeal necrosis, portal inflammation, and fibrosis were seen in the group of the treated with BDL alone. Histo-pathological aspects linked with cholestasis were undermined by AGME although AGME did not significantly reduce the number of bile duct with the BDL group.

CONCLUSION: The protective effect of AGME against the BDL induced liver fibrosis may be mostly connected with the strength of this extract to inhibit TGF-*β*1 and weaken oxidative stress. The ability of AGME to suppress the release of TGF-*β*1 and weaken oxidative stress and benefit of its being naturally derived product as a nontoxic is the main factor that builds this extract a combination therapy for treating and preventing of cirrhosis and human hepatic fibrosis. The presence of antioxidant compounds may be responsible for the effectiveness of AGME against hepatic disorders.

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