

## **STUDY THE EFFECT OF BAY LEAF EXTRACT ON THE SOME BIOCHEMICAL PARAMETERS IN DIABETIC MALE RAT INDUCED BY ALLOXAN**

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### **ABSTRACT**

The present study aimed to determine the ameliorative effect of bay leaf extract(BLE) on some biochemical parameters of laboratory diabetic male rats (*Rattus norvegicus*) induced by Alloxan .The study consisted of thirty adult male rats randomly divided into five equal group(six of each ). As follow :Group one the animals treated by intra peritoneal injection (IP) of normal saline solution (0.25ml),Group two the animal treated by oral gavage with (BLE) at dose (500 mg /Kg/BW) daily .Group three , rats were treated I.P with 100 mg /Kg BW single dose of alloxan(Allox).Group four .The diabetic rats were given orally by oral gavage low dose of ( BLE)(250 mg /Kg/BW ) daily. Group five ,diabetic rats were given by oral gavage high dose of BLE(500 mg /Kg/BW ) daily for 3 weeks.At the end of experiments period ,rats were sacrificed ,blood were collected by cardiac puncture to investigate biochemical parameters which included glucose ,liver enzyme (ALT,AST and ALP ) lipid profile TG , TC ,HDL-c , LDL-c , ,Total serum protein, Blood urea, and Creatinine concentration . Result indicated a significant increased in Glucose,ALT, AST, ALP, TC, TG, LDL-c ,urea, and Creatinineinin diabetic animal whereas HDL-c significantly decreased

## INTRODUCTION

Bay leaf (BL) is a plant of industrial importance, used in foods, drugs, and cosmetics. The dried leaves and essential oils are used extensively in the food industry for seasoning of meat products, soups and fishes. Chemically it has been found to contain sesquiterpene lactones such as 10-epigazaniolide, Gazaniolide, spirafolide, costunolide, Reynosin, santamarine, flavonoidglycosides, essential oil. It has been reported by (1) the BLE has ability to use as wound healing, neuroprotective, antioxidant, antiulcerogenic, anticonvulsant, antimutagenic, antiviral, anticholinergic, antibacterial, antifungal activities (1). Several health benefits have been scientifically investigated, such as its role in lowering blood glucose and LDL cholesterol, increasing HDL cholesterol levels.

Hyperlipidaemia is the current medical as well as social problem, specially associated with diabetes mellitus leading to increase morbidity and mortality. The major risk factor of hyperlipidaemia is associated with atherosclerosis, which predisposes ischemic heart disease and cerebro-vascular disease (2). In type 2 diabetes patients there is mild to moderate hypertriglyceridaemia, low level of HDL, over production of VLDL and the serum total cholesterol is also increased. Alloxan is a hydrophilic and unstable chemical compound with a shape similar to that of glucose that allows its transportation within the cells of animals. This drug is a cytotoxic compound which causes oxidative base. Insulin also plays an important role in the metabolism of lipids. Insulin is potent inhibitor of lipolysis since it inhibits the activity of the hormone sensitive lipase in adipose tissue and suppresses the release of free fatty acids (3). During diabetes, enhanced activity of this enzyme increases lipolysis and releases more free fatty acids into the circulation (4). Increased fatty acid concentration also increases the beta-oxidation of fatty acids producing more acetyl-coA and cholesterol during diabetes (5), damage to nuclear and mitochondrial DNA. It also inhibits pancreatic cancer by selectively destroying pancreatic islet cells. It also inhibits gall bladder cancer. The mechanisms by which Allox monohydrate brings about its diabetic state includes selective destruction of pancreatic insulin secreting  $\beta$ -cells, which make cells less active (6) and lead to poor glucose utilization by tissues (7). Allox has been widely used to produce experimental diabetes mellitus syndrome.

It causes necrosis of pancreatic  $\beta$ -cells and induces free radicals which play a relevant role in the etiology and pathogenesis of both experimental and human diabetes mellitus (8). Moreover, wide spread lipid deposits throughout the exocrine tissue, and loss of  $\beta$ -cells (9).

## **MATERIALS AND METHODS**

The study was carried out on thirty adult male albino rats (*Rattus norvegicus*) weighing  $(250 \pm 25\text{g})$ . They were kept under standard environmental conditions at temperature  $24\text{-}28^\circ\text{C}$  and 12 hr photoperiod. They were acclimatized for 2 weeks before the start of the experiment and housed in polyethylene cages with wire mesh, 2 rats per cage. They were fed standard rat pellets and fresh clean water was provided *ad libitum* throughout the experimental period.

### **Preparation of Bay leaf extract**

Bay leaf (BL) were cleaned, washed and dried at room temperature. Leaf were grounded for 2 minutes by electrical grinder. The leaf powder were refluxed with 250 ml (ethanol 70%) for 12 hours by Soxhlete, and then filtered by using Buchner funnel and filter paper. The solvent was dried and concentrated by using rotary evaporator at  $50^\circ\text{C}$ . The final dryness was done by leaving residue at room temperature.

### **Induction of diabetes mellitus**

Diabetes mellitus (DM) was induced in overnight fasting rat by a single injection of allox (alloxan monohydrate) at dose  $100\text{ mg / kg}$  body weight into intraperitoneal (I.P). Each 100 mg of alloxan was diluted in 1 ml of normal saline (9) after injection allox. Rats were given 5% glucose solution for 24hrs with drinking water to prevent initial drug-induced hypoglycemic mortality (10).

### **Experimental Design:-**

The animal divided randomly into five equal groups (6 rats in each group) as follow:-

**Group one (control):** in which rats were injected I.P ( 0.5 ml )of normal saline daily

**Group two :** in which rats were given by oral gavage (BLE ) at dose(500 mg /Kg/BW ) daily .

**Group three:-** rats were injected I.P 100 mg /Kg BW single dose of allox .

**Group four:** diabetic rats were given orally by oral gavage low dose (250 mg /Kg/BW ) of (BLE) daily .

**Group five:** -diabetic rats were given by oral gavage high dose (500 mg/Kg/BW ) of BLE daily .

#### **Collection of blood samples.**

Blood sample ( 2 ml) were collected from heart puncher , after anaesthetized the rats with chlorophorme . 3 ml of blood collected from each animal were stored in tube without anticoagulant and allowed to clot at room temperature . Then the blood samples were centrifuged for 30 minutes at ( 5000 rpm ) .Serum sample were stored in polyethylene tubes at ( - 20c ) until used for biochemical analysis .

#### **Biochemical test**

The biochemical tests were done in the laboratory by using chemistry auto analyzer made in Germany by human star company serial no.20628 ,the machine has 54 wells which numbered from 1 to 54 , The serum samples deposited in each specific wells . The reagent was put in a special container beside the wells. The serum biochemical parameters estimated by this instrument were lipid profile TG , TC ,HDL , LDL , AST,ALT, ALP, Total serum protein,Blood urea,and SerumCreatinine concentration.

## **RESULTS**

The results in table (1) showed a significant ( $p \leq 0.05$ ) decreased in HDL-c whereas TC, TG and LDL-c were significantly elevated ( $p \leq 0.05$ ) in allox group compared with the control group. The administration of two doses 250 and 500 mg / kg .Bw of BLE after one hour of injection of Alloxan lead to decrease in TC, TG and LDL-c and increased in HDL- c compared to control group .

Serum ALT , AST and ALP Enzyme were increased significantly ( $p \leq 0.05$ ) in Alloxan group compared with control and two doses of BLE 250 and 500 mg/kg.BW table (2) .

Data in this study indicated a significant increase( $p \leq 0.05$ ) in creatinine , urea and glucose in animal treated by alloxan 100 mg / kg . Bw ,whereas the total protein showed a significant ( $p \leq 0.05$ ) decreased compared with control group table(3) . The same table showed that two doses 500 mg / kg.Bw was acted better on urea , creatinine , total protein and glucose value compared to 250 mg /kg Bw day value and reach almost to the normal value .

**Table (1): Effects of BLE and Alloxan on serum lipid profile: (TC), (TG), (HDL), and (LDL). (Mean  $\pm$  SD) n=6**

Treatment Group	TC mg/dL	TG mg/dL	HDL-c mg/dL	LDL-c mg/dL
Control	89.93 $\pm$ 5.14 b	61.39 $\pm$ 4.11 b	23.41 $\pm$ 1.45 b	22.98 $\pm$ 2.13 b
BLE 250 mg/kg	97.73 $\pm$ 6.11 b	58.54 $\pm$ 4.89 b	26.98 $\pm$ 2.65 a	23.28 $\pm$ 2.51 b
Allox 100 mg//kg	188.03 $\pm$ 8.41 a	101.02 $\pm$ 6.8 a	18.87 $\pm$ 1.18 c	77.54 $\pm$ 6.85 a
Allox + BLE 250 mg/kg	88.27 $\pm$ 5.5 b	69.99 $\pm$ 4.99 c	21.55 $\pm$ 2.41 bc	27.03 $\pm$ 3.31 b
Allox 100 mg/kg + BLE 500 mg/kg	85.37 $\pm$ 5.8 b	65.57 $\pm$ 5.11 bc	24.22 $\pm$ 3.05 b	24.3 $\pm$ 1.89 b

The different small letters refer to significant differences at ( $p \leq 0.05$ ) among day

**Table (2): The effects of bay leaf extract and alloxan on ALT . AST and ALP.(Mean  $\pm$  SD ) n=6**

Group	ALT (U/L)	AST (U/L)	ALP (IU/L)
Control	23.54 $\pm$ 2.87 c	17.06 $\pm$ 1.08 c	25.32 $\pm$ 2.48 b
BLE 500 mg/kg	22.93 $\pm$ 1.21 c	16.96 $\pm$ 2.11 c	20.48 $\pm$ 1.87 c
Allox 100 mg//kg	51.77 $\pm$ 4.5 a	54.88 $\pm$ 4.45 a	34.98 $\pm$ 3.41 a
Allox 100 mg/kg + BLE 250 mg/kg	26.84 $\pm$ 1.89 b	22.74 $\pm$ 2.53 b	22.53 $\pm$ 2.32 c
Allox 100 mg/kg + BLE 500 mg/kg	24.13 $\pm$ 2.14 b	21.82 $\pm$ 1.81 b	24.676 $\pm$ 2.11 b

The different small letters refer to significant differences at ( $p \leq 0.05$ ) among day treatment

**Table (3): The effects of bay leaf and alloxan on Urea ,Createnin ,protein and glucose(Mean  $\pm$  SD ) n=6**

Group	Urea mg/dL	Creatinen mg/dL	Protein g/dL	glucose mg / dl
Control	22.65 $\pm$ 2.18 b	0.53 $\pm$ 0.09 b	4.23 $\pm$ 0.81 a	115.97 $\pm$ 6.81 B
BLE 250 mg/kg	22.72 $\pm$ 3.15 b	0.30 $\pm$ 0.07 c	3.91 $\pm$ 0.78 b	95.91 $\pm$ 7.71 c
Allox 100 mg//kg	37.54 $\pm$ 3.41 a	3.04 $\pm$ 0.48 a	2.69 $\pm$ 0.98 c	424.45 $\pm$ 10.14 a
Allox 100 mg/kg + BLE 150 mg/kg	21.55 $\pm$ 2.89 b	0.39 $\pm$ 0.08 c	3.83 $\pm$ 0.83 b	103.50 $\pm$ 6.81 bd
Allox 100 mg/kg + BLE 250 mg/kg	24.59 $\pm$ 2.41 b	0.53 $\pm$ 0.02 b	4.53 $\pm$ 0.34 a	93.34 $\pm$ 4.89 d

The different small letters refer to significant differences at ( $p \leq 0.05$ ) among day treatment

## DISCUSSION

It seems from the that an significant elevated in serum lipid level observed in the alloxan group induced diabetic when compared to BLE and control group,the result may be due to disturbance in the regulation of the activity of the hormone-sensitive enzyme, lipase, by insulin due to its deficiency or absence, caused by the Alloxan induced destruction of beta islet cells in pancreas. Lipase is known to convert triglycerides to free fatty acids and glycerol. Insulin inhibits the hormone -sensitive lipase in adipose tissue and in the absence of insulin, the plasma level of free fatty acids increases. In liver, the free fatty acids are catabolized to acetyl CoA, and the excess acetyl CoA is converted to cholesterol, triglyceride and ketone bodies resulting in ketosis (11). The abnormally high concentration of serum lipoprotein in the Alloxan rats may also be due to increase in the mobilization of free fatty acids from the peripheral fat depots by glucagon in the absence of insulin(12). Excess of fatty acids in plasma produced by the alloxan-induced diabetes promotes the liver conversion of some fatty acids into triacylglycerol, phospholipids and cholesterol which may be discharged into the blood as lipoproteins (11) The treatment with the plant (BLE) resulted in decrease in plasma cholesterol, TG and LDL level with increase in HDL level the result agreement with (12). they revealed that the extract exerts no toxic effect on the monolayer hepatocyte layer . The significant ( $p<0.005$ ) hypolipidemic activities shown by the BLE administered orally in two doses when compared to Alloxan group might be due to ability of the methanolic extract of BL caused regeneration of the  $\beta$ -cells of the pancreas and potentiation of insulin secretion from surviving  $\beta$ -cells , the increase in insulin secretion and the consequent decrease in blood glucose level may led to stimulate of fatty acid biosynthesis (Insulin stimulates lipid synthesizing enzymes (fatty acid synthase, acetyl-CoA carboxylase) and also the incorporation of fatty acids into triglycerides in the liver and adipose tissue). In the presence of insulin, the hormone-sensitive lipase will be inhibited in the adipose tissue, and mobilization of fatty acid from adipose tissue by glucagon will also be inhibited and therefore leading to the observed decrease plasma level of free fatty acids.

ALT enzyme is synthesized intra cellular in almost all tissues of the body, but most of its amount found in the liver, the amount of this enzyme found in small amount into the blood stream. If the damage of liver occurred this enzyme released into the blood stream and as a result of destruction of these cells, this increased level can be determined by laboratory methods. AST enzyme is also a biochemical marker for the diagnosis of myocardial injury. Increased AST and ALT in the blood plasma of experimental animals may indicate damaging in liver cell by effect of the Alloxan (13). Serum liver enzyme AST ,ALT and ALP in table (2) significantly increased in blood serum in animal treated Alloxan ,the results agreement with (14,15) A study conducted showed clinical and experimental evidence suggests that diabetic mellitus (DM) affects the liver in addition to blood vessels, However, the recognition of DM as the primary cause of chronic liver disease is neglected in medical practice because of the wide variety of clinical, metabolic and hormonal conditions that can lead obesity, malnutrition, intestinal malabsorption, dyslipidemia, thyroid disorders, and metabolic syndrome (16). Hyperglycemia increases the generation of free radicals by glucose auto oxidation and the increment of free radicals may lead to liver cell damage. Another finding may be noted in this study is the elevation of AST, ALT and ALP activities in diabetic rats. Elevated serum levels of AST and ALT usually indicate hepatocyte damage and the most common presentation is elevated liver enzymes AST and ALT in fatty liver .The elevation of the activities of liver enzymes in plasma may be mainly due to the leakage of these enzymes from liver cytosol to blood stream which gives an indication on hepatotoxic effect of Alloxan (17). The result in group four and five showed decreased ALT, AST and ALP enzyme level that maybe due to hepatoprotective properties of the bay leaf extract. This is probably due to the presence and combined action of the extract phytochemicals which have flavonoid nonflavonoid origin such as, terpenes and terpenoids possessing antioxidative and antimicrobial activities (18).

Allox caused diabetic rats showed a significant increase in urea, total protein and creatinine compared to control group. In comparison with control group, intake of cinnamon significantly decreased the levels of these parameters in diabetic rats. It was obvious that, the recovery of these parameters was better in composite diet treated group after 3 weeks .

Impairment of kidney function is a prominent feature of diabetes, therefore the elevated levels of urea, uric acid and creatinine were shown in diabetic rats, maybe due to kidney failure. These results were in accordance with (19) who found that the animals treated with allox lead to increase in urea and creatinine .

The BLE significantly decreased the elevations in these parameters, suggesting composite diet of BLE may play an important role in improving impaired kidney function. It can be suggested that the active anti hyperglycemic agents present in the composite diet of BLE helps in overcoming the diabetic complications by increasing the insulin secretion or by scavenging free radicals and preventing the depletion of endogenous antioxidants.(20) ,or by the BLE like BL contain various phenolics antioxidants such as flavonoids, rosmarinic acid (RA), tannins, coumarins, xanthenes, and procyanidins have been shown to scavenge radicals in a dose dependent manner . It has been known that BLE exhibits in tend to increase the amount of proteins involved in insulin signaling and glucose transport (21). Many studies have demonstrated that BLE , and its active constituent cinnamaldehyde, doses dependently improve glycemic control in normal and Allox diabetic rats (22)

### دراسة تأثير مستخلص ورق الغار على بعض المعايير الكيموحيوية في الجرذان المستحث بها داء السكر بواسطة الالوكسان

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#### الخلاصة

تهدف الدراسة الحالية لتقييم الدور المحسن لمستخلص ورق الغار ولمدة ٢١ يوم تجريع لذكور الفئران المختبرية من خلال دراسة بعض الفحوصات البايوكيميائية:-.

تضمنت الدراسة ٣٠ من ذكور الجرذان والتي قسمت عشوائيا الى خمس مجاميع ( ستة حيوانات في كل مجموعة ) . المجموعة الاولى تمثلت بمجموعة السيطرة والتي حققت بالمحلول الفسيولوجي والمجموعة الثانية جرعت بمستخلص ورق الغار جرعة ٥٠٠ ملغم لكل كغم من وزن الجسم والمجموعة الثالثة حققت بمادة الالوكسان لغرض استحداث السكر ، جرعة واحدة ١٠٠ ملغم لكل كغم من وزن الجسم والمجموعة الرابعة جرعت الحيوانات المستحدث السكر فيها بجرعة واطئة من مستخلص ورق الغار ٢٥٠ ملغم لكل كغم من وزن

الجسم والمجموعة الخامسة جرعت الحيوانات المستحدث السكر فيها بجرعة عالية من مستخلص ورق الغار ٥٠٠ ملغم لكل كغم من وزن الجسم .

في نهاية التجربة، والتي استمرت احدى وعشرون يوم تم قتل الحيوانات وجمع الدم من القلب مباشرة لدراسة التغيرات اليايوكيميائية متمثلا بقياس معدل سكر الدم وانزيمات الكبد ومستوى الدهون ووظائف الكلية . اظهرت النتائج وجود ارتفاع معنوي واضح في معدل سكر الدم في الحيوانات المستحدث السكر فيها وكذلك ارتفاع بمستوى الدهون متمثلة بالكوليسترول والدهون الثلاثية ومستوى البروتين الكلي في الجسم و-LDL ومستوى اليوريا والكرياتينين في مجموعة الحيوانات المستحدث بها السكر بواسطة الالوكسان بينما اظهرت النتائج انخفاض معنوي واضح بمستوى HDL-C لنفس المعاملة . ادت المعاملة بمستخلص ورق الغار الى رجوع القيم اعلاه الى او قريبة الى مجموعة السيطرة .

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