



Effect of aqueous extract of black pepper and ajwa seed on liver enzymes in alloxan-induced diabetic Wister albino rats



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ABSTRACT

The aim of the present study was to investigate the effects of aqueous extract of black pepper and ajwa seed on liver enzymes in alloxan-induced diabetic Wister albino rats to show the preventive and ameliorating effects in hyperglycemic rats. Rats were divided into 6 groups; normal control rats, diabetic control rats, glibenclamide treated rats, black pepper treated rats, ajwa seed treated rats and black pepper plus ajwa seed treated rats. Hyperglycemia was induced in the treatments groups by a single intraperitoneal injection of alloxan at 150 mg/kg body weight. The extracts were administered via oral incubation, doses were glibenclamide 10 mg/kg, black pepper 50 mg/kg, ajwa seed 500 mg/kg and their mixture 500 mg/kg body weight for a period of 8 weeks. Serum glucose, AST, ALT and ALP were assayed using spectrophotometric method. Results showed that ajwa seed and mixture significantly reduced glucose level. AST level was significantly reduced by mixture treated group. No significant difference was observed between different aqueous extract treated group in ALT and ALP level. The study indicates that black pepper and ajwa seed extract to some extent normalized the glucose and liver enzyme activities in alloxanized diabetic rats.

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1. Introduction

Diabetes mellitus has become a global problem in recent decades: it is a leading cause of blindness, end stage renal disease and non-traumatic amputation. Its prevalence increased day by day worldwide without any plateau (Spanou and Tziomalos, 2013; Nawaz et al., 2017a,b). So, there is need of healthy natural products to cure this disease (Abbas et al., 2017; Gohar et al., 2017). Date fruit (*Phoenix dactylifera* L.) as a source of nutrition and economics has become important in few countries (Briones et al., 2011). *Phoenix dactylifera* L. belongs to the Arecaceae family. Dates pits are more valuable due to their nutritional composition and high amounts of dietary fibers present in date pits (Bouaziz et al., 2010). The seed powder is used in few traditional medicine due to potential of human health benefits. Date palm possess antioxidant, anticancer, antihypertensive, antimutagenic,

antifungal and antidiarrheal potential (Janbaz et al., 2013). Black pepper (*Piper Nigrum*) belongs to family Piperaceae, a perennial plant producing berry-like and pungent fruit. It is cultivated in many tropical regions but it still occurs wild in the mountains of Kerala state in India. It is valued due to its different bitter quality credited to piperine and its isomers. Black pepper possesses immune-stimulatory, anti-tumorigenic, carminative, anticholesterolemic and also known for its phytochemical activities. Piperine helps in the absorption of various substances like vitamins, curcumin and other nutrients (Ahmad et al., 2010; Muhammad et al., 2017). Due to their valuable uses the present study aimed to evaluate the black pepper and ajwa seed extract on liver enzymes activities.

2. Materials and methods

The research was carried out in the Institute of Pharmacy, Physiology and Pharmacology, University of Agriculture Faisalabad, Pakistan.

2.1. Collection and preparation of extract

Black pepper and ajwa date were purchased from market and was identified and authenticated by taxonomist at Department of

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Botany, University of Agriculture, Faisalabad. Seeds were separated from ajwa date. The collected plant material was washed with tap water carefully and was completely dried under the shade. The shade-dried plant material was finely powdered. The aqueous extracts were prepared by soaking about 100 g of powdered plant material in 1000 ml water for 24 hours. The extract was filtered and the solvents removed with rotary evaporations to obtain crude active ingredient.

2.2. Experimental animal

Male Wistar albino rats (180–200 g) were used for the antidiabetic evaluation. The animals were used from the stock of animal house of Institute of Pharmacy, Physiology and Pharmacology, University of Agriculture, Faisalabad. The rats were housed in laboratory conditions in stainless steel cages at standard conditions (temperature 24 ± 2 °C, relative humidity 45–55% and 12 h light/dark cycle). They were provided with commercial rat feed and water *ad libitum*.

2.3. Induction of diabetes mellitus

In overnight fasted rats, diabetes was induced by single intraperitoneal injection of alloxan monohydrate (150 mg/kg) dissolved in normal saline. Diabetes was confirmed in rats by determining glucose level with a glucometer after 72 hrs of alloxan monohydrate injection. Experimental rats having blood glucose level above 300 mg/dl were believed to be diabetic and included for further studies.

2.4. Experimental design

The study was carried out for eight weeks. The rats were acclimatized for one week and maintained standard laboratory conditions. Diabetes was induced in rats a week before the start of the experiment. After the induction of diabetes, they were divided into different groups. Varying concentrations of the crude extracts of black pepper, ajwa seed and their mixture were administered via oral intubation to the animals. Blood samples were collected and serum was separated for glucose and liver enzymes estimation.

Group 1: normal control (C) rats

Group 2: diabetic control rats (Positive control)

Group 3: Diabetic rats given Glibenclamide (G) (10 mg/kg body weight)

Group 4: Diabetic rats given black pepper (BP) (50 mg/kg body weight)

Group 5: Diabetic rats given ajwa seed (AS) (500 mg/kg body weight)

Group 6: Diabetic rats given BP + AS (500 mg/kg body weight)

2.5. Assays

The glucose level was determined by GOD-POD estimation kit. Serum aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) activities were estimated with the Randox reagent kit using 2, 4-dinitrophenylhydrazine as substrate according to the method described by [Reitman and Frankel \(1957\)](#).

2.6. Statistical analysis

All the results obtained were expressed as mean \pm S.E. Data will be analyzed statistically by analysis of variance, for statistical significance using DMR test, one way ANOVA. Differences between means were regarded significant at $P < 0.05$.

3. Results

3.1. Effect of aqueous extract of black pepper, ajwa seed and their mixture on serum glucose level of diabetic rats

Fig. 1 shows the effect of aqueous extract of black pepper, ajwa seed and their mixture on serum glucose level of rats after treatment of diabetes. There was a significant ($P < 0.05$) increase in glucose level in positive control group when compared to normal control group indicating that alloxan causes hyperglycemia in rats but the administration of ajwa seed and their mixture significantly ($P < 0.05$) decreased the glucose level. There is no significant difference when compared to glibenclamide.

3.2. Effect of aqueous extract of black pepper, ajwa seed and their mixture on serum aspartate transaminase (AST) level of diabetic rats

Fig. 2 shows the effect of aqueous extract of black pepper, ajwa seed and their mixture on serum aspartate transaminase level of rats after treatment of diabetes. There was a significant ($P < 0.05$) increase in AST level in positive control group when compared to normal control group. Mixture treated group significantly ($P < 0.05$) reduced AST level as compared to black pepper. While no significant ($P < 0.05$) was observed between glibenclamide and mixture treated group.

3.3. Effect of aqueous extract of black pepper, ajwa seed and their mixture on serum alanine transaminase (ALT) level of diabetic rats

Fig. 3 shows the effect of aqueous extract of black pepper, ajwa seed and their mixture on serum alanine transaminase level of rats after treatment of diabetes. There was a significant ($P < 0.05$) increase in ALT level in positive control group when compared to normal control group. No significant ($P < 0.05$) difference was observed between different treated groups as compared to standard drug glibenclamide but significantly ($P < 0.05$) reduced ALT level as compared to positive control group.

3.4. Effect of aqueous extract of black pepper, ajwa seed and their mixture on serum alkaline phosphatase (ALP) level of diabetic rats

Fig. 4 shows the effect of aqueous extract of black pepper, ajwa seed and their mixture on serum alkaline phosphatase level of rats after treatment of diabetes. ALP level was significantly ($P < 0.05$) increased in diabetic control group as compared to normal control. No significant ($P < 0.05$) difference was observed between different treated groups as compared to glibenclamide and diabetic control group.

4. Discussion

Alloxan induced diabetic rats are one of the animal model for diabetes. It is well known for pancreatic islets beta cell cytotoxicity and commonly used to induce diabetes. Alloxan causes time and concentration dependent degradation lesions of the pancreatic beta cells leading to hyperglycemia. A standard antidiabetic drug glibenclamide is most frequently used in alloxan-induced diabetes to compare the efficacy of variety of hypoglycemic agents ([Baldi and Goyal, 2011](#); [Nawaz et al., 2017a,b](#)).

Ajwa date has increased acceptance as life-saving nutritional power plant that can feed the needy. It is commonly used to treat cardiac disorders and infections as a natural therapy. Findings of our study showed that ajwa seed extract and the mixture of black pepper and ajwa seed produced a marked decrease in blood glucose level in diabetic rats after 8 weeks of treatment. The

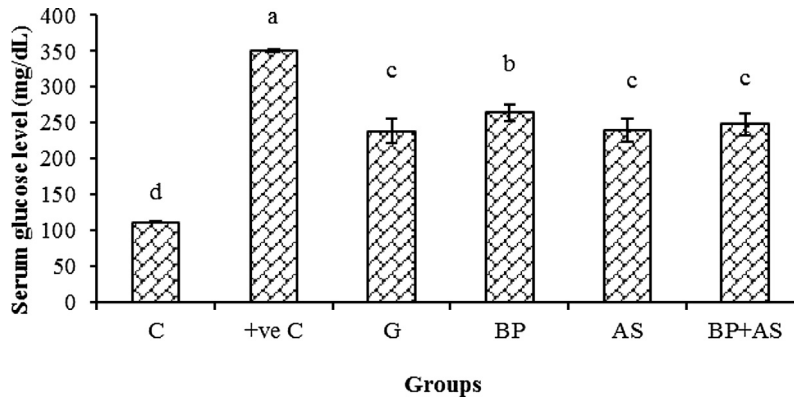


Fig. 1. The effect of aqueous extract of black pepper (BP), ajwa seed (AS), their mixture (BP + AS) and glibenclamide (G) on serum glucose level of diabetic rats.

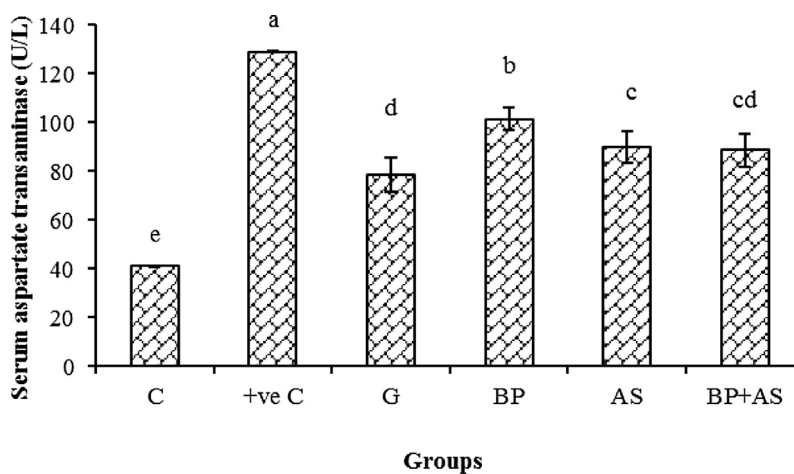


Fig. 2. The effect of aqueous extract of black pepper, ajwa seed, their mixture and glibenclamide on serum aspartate transaminase (AST) level of diabetic rats.

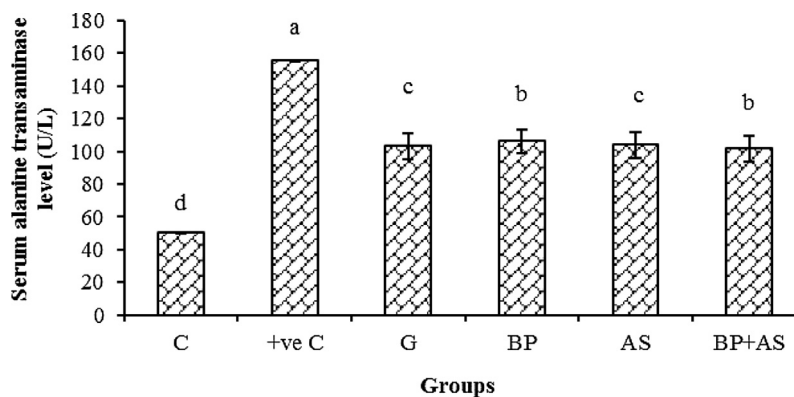


Fig. 3. The effect of aqueous extract of black pepper, ajwa seed, their mixture and glibenclamide on serum alanine transaminase (ALT) level of diabetic rats.

antidiabetic effect may be due to release of insulin from existing pancreatic beta cells. Our findings agree with those reported by El Fouhil et al. (2013) and Manoharan et al. (2007).

In diabetic animal's alteration in the activity of serum enzymes directly related to changed metabolism in which enzymes are involved. Increased activity of liver enzymes in diabetic animals are reported by many researchers. Increased transaminases level in absence of insulin because of increased amino acid activity in hyperglycemic condition are responsible for ketogenesis and gluconeogenesis (Baldi and Goyal, 2011; Atta et al., 2017).

The present study represents increased activities of serum AST, ALT and ALP level indicated that hepatic dysfunction may be induced due to hyperglycemia in diabetic rats. The best results of liver function recorded with group fed by ajwa seed aqueous extract they showed no significant difference with standard drug glibenclamide. The results of the present study agree with Reda (2006), Najafi (2011) who reported that extract of date seed shows capability to restore the normal functional status and also to protect liver in rats against alloxan induced toxicity. Moreover, in another study it was documented that by improving the diabetic

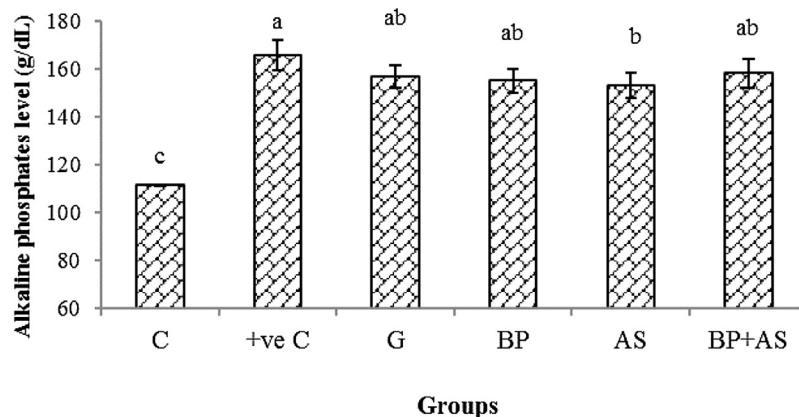


Fig. 4. The effect of aqueous extract of black pepper, ajwa seed, their mixture and glibenclamide on serum alkaline phosphatase (ALP) level of diabetic rats.

diet helps to prevent diabetic complications (Hadrami and Al-Khayri, 2012; Ishaq and Jafri, 2017; Mustafa et al., 2017). Wakade et al. (2008) reported in their study that black pepper decreased significantly AST level in diabetic rats but there is non-significant effect observed at ALT and ALP level. Along with that Salih (2013) reported in their study that individuals with diabetes are at a high risk of liver function abnormalities as compare to normal. Slight chronic elevation in transaminases level most often reflect the insulin resistance.

5. Conclusion

Diabetes mellitus has been a serious disease all over the world. Plants play an important role for the treatment of this disease due to their active constituents. The present study indicates that black pepper and ajwa seed extract to some extent normalized the glucose level and liver enzyme activities in alloxanized diabetic rats and it could be used as an adjunct in the management of hyperglycemic conditions.

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