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## Alpha Amylase Enzyme Inhibitory and Anti-inflammatory Effect of *Lawsonia inermis*

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**Abstract:** Previously it was reported elsewhere that *Lawsonia inermis* have anti-inflammatory and analgesic effect in experimental animals. The *in vitro* porcine alpha amylase inhibitory effect was investigated of this plant methanolic extracts and consequently hypoglycemic effect by quantitatively determining the maltose from the maltose standard curve while the anti-inflammatory effect by acetic acid induced writhing test in mice. Acarbose ( $10 \mu\text{g mL}^{-1}$ ) and Diclofenac sodium ( $20 \text{mg kg}^{-1}$ ) were used as reference hypoglycemic and anti-inflammatory drugs, respectively, for this study. The methanolic leaves extract of the plant significantly inhibited (60.97% compared to untreated) enzymatic activity of the amylase at  $10 \mu\text{g mL}^{-1}$  dose ( $p < 0.05$ ) also reduced the chemically induced nociceptive pain stimuli significantly at all doses ( $p < 0.01$ ). Carbohydrates, glycosides, flavonoids, saponins and tannins were found to have in phytochemical screening of the extract which are thought to bring these effects. For the conclusive purpose, it is suggesting from the result that the pharmacological properties of this *Lawsonia inermis* can elicit hypoglycemic effect by inhibiting  $\alpha$ -amylase enzyme and can reduce neurogenic pain stimulus. It gives the notion that how this group of patient would be therapeutically benefitted by decreasing both these effects by the same agent which is easy available.

**Key words:** Hypoglycemia, alpha amylase, inflammation, *Lawsonia inermis*

### INTRODUCTION

The prevalence of type 2 diabetes mellitus in Bangladesh is vastly increasing. Many evidences suggesting the association between type 2 diabetes and inflammation (Wellen and Hotamisligil, 2005). It is reported that hyperglycemia induce Reactive Oxygen Species (ROS) production in the adipocytes and consequently proinflammatory cytokines release (Lin *et al.*, 2005). Also shown, the type 2 diabetes caused by insulin resistance in obese people, is primarily linked with some proinflammatory cytokines (Wellen and Hotamisligil, 2005). These studies are vividly showing the close relationship between hyperglycemia and inflammation. Hence, it seems to be very effective therapy to reduce both hyperglycemia and inflammatory mediators together in type II diabetes.

A lot of commercially available drugs are in the market now in order to treat both hyperglycemia and inflammation. Furthermore some negative clinical outcomes are observed have led to the investigation of new therapeutic approaches focused on controlling postprandial glucose levels. Pancreatic  $\alpha$ -amylase (E.C value 3.2.1.1) is a key enzyme for initial catalysis of

carbohydrate to maltose and consequently glucose formation. This postprandial glucose level can be reduced by inhibiting the  $\alpha$ -amylase (Ceriello, 2005) and it will possibly be the chief and realistic approach to inhibit the enzyme associated with carbohydrate metabolism. On the other hand, management of inflammatory pain with steroids and other anti-inflammatory drugs sometimes cause with side effects. So, there is constant demand for alternatives.

Many ongoing research has reported the efficacy of plant extracts in inhibiting the alpha amylase in their *in vitro* study. Leaves, fruits, roots, barks and many other parts of different plant species have shown with potential inhibitory effect on this enzyme and to reduce the postprandial hyperglycemia, but still it is not enough to find the potential candidate with highest efficacy and many plant is on the pipeline to be evaluated. One such therapeutically useful plant *Lawsonia inermis* also commonly known as henna which has a wide therapeutic description in Indian Ayurvedic or natural herbal medicines (Lavhale and Mishra, 2007). Henna leave extracts have shown to possess anti-inflammatory (Alia *et al.*, 1995; Gupta *et al.*, 1986), anti-fungal (Singh and Pandley, 1989), anti-pyretic and analgesic

(Alia *et al.*, 1995), anti-oxidant and immunomodulatory (Mikhaeil *et al.*, 2004) and antibacterial (Ali *et al.*, 2001) activities.

The purpose of this study was to investigate the *in vitro* porcine  $\alpha$ -amylase enzyme inhibitory effect and *in vivo* anti-inflammatory effect of methanolic henna leaves extract.

## MATERIALS AND METHODS

### Identification of plant and preparation of extract:

*Lawsonia inermis* leaves were collected in August from Nikunjo, Dhaka, Bangladesh after botanically identified and authenticated. It was then properly washed with tap water and air dried around 20 days. Dried leaves were powdered using mortar pestle and 60 g powder was soaked in 300 mL methanol for 3 days with occasional stirring. Filtrate was prepared by filtering through cotton and Whatman filter paper and finally centrifugation. Solvent was evaporated using a vacuum evaporator and reduced to 4.1% w/w which was preserved in -18°C until experiments was done by weighing for desired concentration in water as gram per liter.

**Experimental animal:** Swiss Albino mice were collected from ICDDRDB and housed in plastic case of (28×22×13 cm) dimensions. Soft wood shavings were served as their bedding in side the cages. Standard condition (24±1°C temperature and 55-65% relative humidity, 12 h dark/12 h light) was maintained in the animal house and standard food pellets were supplied *ad libitum*.

**Alpha amylase inhibition assay:** *In vitro* amylase inhibition assay was done by following the chromogenic DNSA method described by Miller (1959), with the following modification. Briefly, 20  $\mu$ L sample solution was mixed with 1500  $\mu$ L enzyme solution (8 unit, calculated by maltose standard curve preparation, data not shown) in buffer containing 50 mM Tris-HCl, 20 mM NaCl and 2 mM CaCl<sub>2</sub> at a pH of 7.0. Following mild vortexing, 500  $\mu$ L of 1% starch solution was added and set the mixture for 5 min at 37°C and reaction was stopped by adding 50  $\mu$ L of 1 N HCl. Blank was prepared by adding 50  $\mu$ L of 1 N HCl in the buffer solution. These solutions were mixed with 1 mL DNSA reagent and boiled for 15 min at 100°C in water bath and cooled at room temperature for 10 min and the absorbance was measured at 540 nm. Acarbose (10  $\mu$ g mL<sup>-1</sup>) was used as a positive control. The % of  $\alpha$ -amylase inhibition activity was measured by the following formula:

$$\text{Inhibition of enzymatic activity (\%)} = \frac{\text{Control-test}}{\text{Control}} \times 100$$

**Anti-inflammatory Activity of *Lawsonia inermis*:** Acetic acid induced writhing test is long been used to test the analgesic anti-inflammatory properties for new agents (Collier *et al.*, 1968). It consists of first feeding the anti-inflammatory agent or vehicle only as a negative control followed by intra peritoneal administration of 0.6% acetic acid (0.45 mL/mouse), 30 min later. It causes a painful inflammatory effect. The painful sensation of mouse which was manifested as abdominal writhe, were counted after the acetic acid injection.

**Maltose standard curve preparation:** Standard maltose curve was constructed in order to measure quantitatively the disaccharide formation from the starch in the presence of amylase (data not shown). Standard maltose concentration of 20, 30, 40 and 50  $\mu$ g mL<sup>-1</sup> was prepared by dilution in water. Unknown disaccharide concentration was calculated by the equation  $y = mx + c$ . In this equation, “y” and “x” are the absorbance of sample and the concentration respectively. Whereas, “m” and “c” representing the slope and y-intercept of that equation.

**Statistical analysis:** Each data point represents the average of three individual experiments and each time duplicate samples were there. Statistical analysis was performed using Microsoft Excel 2007 and data were compared with unpaired two tailed t-test where p-value threshold was used  $\leq 0.05$  to indicate a statistical significant.

## RESULTS AND DISCUSSION

Previously it was reported elsewhere that, in type II diabetes, hyperglycemia and inflammatory mediators are interrelated. For its ethno medicinal efficacies, henna leaves, steams, barks, seed and root are being used for many centuries in different ailments. Still many concealed therapeutic potential need to be evaluated. Therefore, the postprandial glucose level reduction effect through porcine  $\alpha$ -amylase enzyme inhibition in the *in vitro* model together the nociceptive pain reduction effect in mouse model by acetic acid induced writhing test assay was investigated of the leaf methanolic extract of *L. inermis* because of its conventional many folkloric therapeutic uses.

*Lawsonia inermis* extracts showed porcine enzyme inhibitory activity at an array of concentrations. In Fig. 1, it showed 29.63, 53.28, 60.97, 48.12 and 57.83 % reduction of enzymatic activity at 5, 7.5, 10, 12.5 and 15  $\mu$ g mL<sup>-1</sup> concentrations of *Lawsonia inermis* extract, respectively. Untreated one was considered as 100% enzymatic activity. Similarly the disaccharide formation, measured by

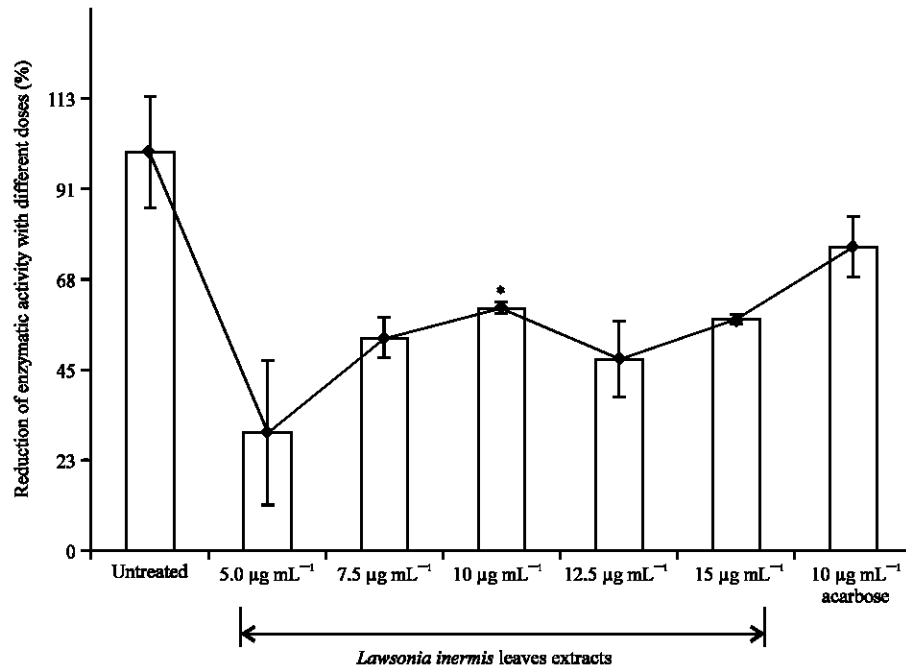


Fig. 1: Percentage of  $\alpha$ -amylase inhibitory activity by acarbose as a standard drug and the methanolic extract of *L. inermis*. Values are Mean $\pm$ SEM \*p-value is 0.05 for 10  $\mu\text{g mL}^{-1}$  concentration of extract using unpaired two tailed t-test vs no extracts (n = 3)

Table 1: Table containing the average concentration of maltose formation, calculated by constructing standard curve with standard error mean and the enzymatic activity in the presence of different dose of henna leave extracts. Acarbose, standard drug was used as a positive control

Henna concentration ( $\mu\text{g mL}^{-1}$ )	Avg. concentration of Maltose ( $\mu\text{g mL}^{-1}$ ) $\pm$ SEM	% Enzymatic Activity
5.00	88.72 $\pm$ 87.04	70.38
7.50	58.27 $\pm$ 17.86	46.72
10.00	47.78 $\pm$ 4.490	39.03
12.05	65.01 $\pm$ 33.26	51.88
15.00	52.47 $\pm$ 3.520	42.17
Acarbose (10 $\mu\text{g mL}^{-1}$ )	31.48 $\pm$ 5.33	24.70

maltose standard curve, was found to have linearly decreased order upto 10  $\mu\text{g mL}^{-1}$  (Table 1) with the increase of inhibitor concentration and the lowest amount of maltose 47.78  $\mu\text{g mL}^{-1}$  was shown in the presence of 10  $\mu\text{g mL}^{-1}$  of inhibitor concentration. Duplicate sample were there and result were steady for each experiment. Acarbose, standard drug for  $\alpha$ -amylase enzyme inhibition, was used as a positive control. Acarbose, at 10  $\mu\text{g mL}^{-1}$  concentration, reduced the enzymatic activity by 76.30% compared to untreated sample.

*Lawsonia inermis*, on the other hand in Fig. 2, acetic acid induced writhing test, showed 16.33, 14, 11.66, 11 times mean writhing at the doses of 200, 300, 400 and 500  $\text{mg kg}^{-1}$  of the extract, respectively. Diclofenac treated group was termed as positive control, that showed 5 mean

writhing at a dose 20  $\text{mg kg}^{-1}$ . In contrast, negative control, was treated with 0.9% saline, showed 20.33 times mean writhing. Each group consists of three male Swiss Albino mice of 4-5 weeks age and weight range from 20-25 g.

The LD<sub>50</sub> value of the aqueous extract of *L. inermis* was found quite high, 894  $\text{mg kg}^{-1}$  in mice (Bello *et al.*, 2010) which shows that the treated doses as an  $\alpha$ -amylase enzyme inhibitor and anti-inflammatory drug of this extract are quite safe.

*Lawsonia inermis* methanolic extract reduced the porcine  $\alpha$ -amylase enzyme activity in all of the concentrations in this experimental setup. At 5% threshold level it gave statistically significant result in 10  $\mu\text{g mL}^{-1}$  concentration. Reduction in enzymatic effect was present in all the doses but it was not concentration dependent. Result was reproducible, was steady for three days. Managing type II diabetes by inhibiting that enzyme with this extract can be a safe and cost effective means, especially in developing countries like in Bangladesh, where type II diabetes is a much prevalent disease.

On the other hand, *Lawsonia inermis* extract was found to have its anti-inflammatory effect too. It was previously been reported the anti-inflammatory activity (Alia *et al.*, 1995; Gupta *et al.*, 1986) and this study also

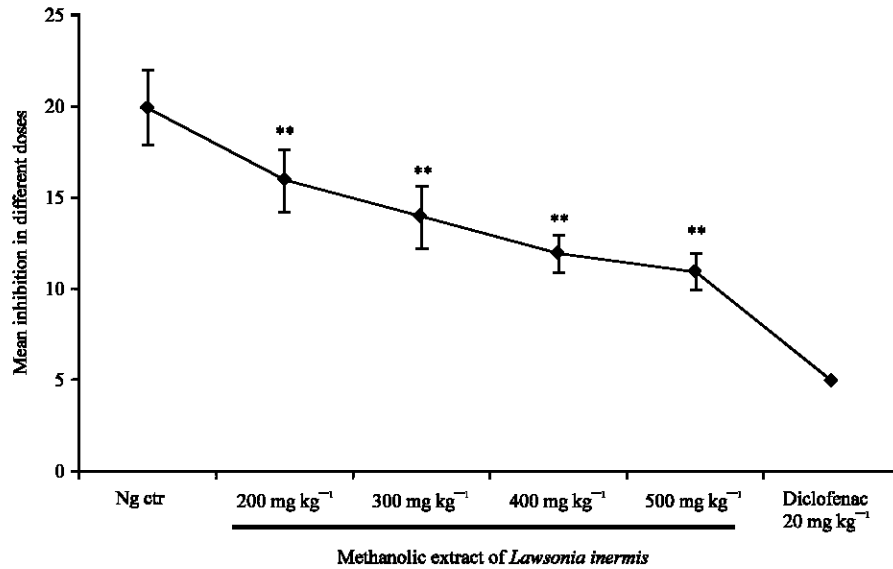


Fig. 2: Mean inhibition in acetic acid induced writhing test with Diclofenac sodium as standard drug for positive control and different doses treatment of methanolic extract of *L. inermis*. All the concentrations of this extract were shown significantly different result compared to untreated one. Values are Means±SEM of three individual observations. \*\*p<0.01, as compared with the control (Two tailed student t-test). Concentration of disaccharide (maltose) formation in different sample

found similar data. Its inflammation reducing capacity is quite impressive. All the concentration of this extract reduced the acetic acid induced writhing and was statistically significant at a 1% threshold level. The trend of anti-inflammatory effect of this extract was concentration dependent that means with increasing dose it reduced the writhing.

Phytochemical analysis was also carried out (data not shown) to determine the chemical constituents present in methanolic extract of the *Lawsonia inermis* leaves. Carbohydrates, glycosides, flavonoids, saponins and tannins were positive in that phytochemical analysis but not alkaloids, in the methanol extract of the leaves. These chemical constituents may be attributed those effects.

It is very essential for diabetic patient to do more physical exercise but it will be an obstacle, if they suffer from different types of arthritis. Where this plant extract were shown with pharmacological aspects-hypoglycemic and reduction of painful stimuli which is frequently encountered in diabetic patients. The methanolic extract of henna reduced both hyperglycemia and inflammation significantly in this experimental setup. Other researchers have already been shown the anti-inflammatory activity (Alia *et al.*, 1995; Gupta *et al.*, 1986) of henna leaves extract, using other methods, thereby reduced experimental bias is proved to be by these cross matched results. But there was no study regarding the alpha

amylase enzyme inhibitory activity of this plant extract. So, this is for the first time reporting of this alpha amylase inhibitory effect of this plant.

Many pharmacological studies showed the spectrum of activity of *Lawsonia inermis*. This versatile medicinal plant contains many chemical compounds with potential therapeutic effect. Hence, extensive investigation is needed for its therapeutic utility.

### CONCLUSION

In the present study, it is found that the methanolic leaves extract of *Lawsonia inermis* effectively reduced the porcine alpha amylase enzyme and thereby reduced the disaccharide formation also effectively reduced the nociceptive pain induced by acetic acid. These pharmacological properties can attribute the folkloric use of the plant in the management and control of hyperglycemia and arthritic pain. Further investigation in human is essential to reveal its therapeutic potential.

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