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Natural aspirin-like compounds from white willow (Salix alba) bark extract prevent structural changes of human hemoglobin during in vitro non-enzymatic glycation and fructation, preserving its peroxidase and esterase activity

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Abstract: Proteins undergo continuous changes under the action of various intrinsic and extrinsic factors, leading to alteration of several intracellular metabolic pathways and the development of various clinical disorders. Although there are currently many effective therapies in the prevention and treatment of these diseases, in the last decade there has been an increasing trend of replacing synthetic drugs by natural compounds, in order to reduce the side effects that may occur, and the production costs. It is well known that aspirin (acetylsalicylic acid) inhibits the glycation process of serum proteins by acetylating N-terminal amino groups and lysine residues in their structure. Therefore, the main purpose of our research was to analyze the non-enzymatic glycation and fructation process of hemoglobin through spectrometric and electrophoretic techniques, in order to reveal how this process could influence the three-dimensional structure and biological function of the protein, and the effect of some natural aspirin-like compounds on the peroxidase and esterase activity of hemoglobin during fructose and glucose binding. Our data indicated that salicylic compounds possess strong antioxidant properties, which give them the ability to participate in the glycosylation process to block the formation of advanced glycation end products (AGEs). Therefore, they can be successfully used as substitutes for aspirin, one of the main synthetic compounds with anti-inflammatory and anti-glycosylating role.

Keywords: hemoglobin; Salix alba; anti-glycation; salicylic compounds

RESULTS AND DISCUSSION

Physicochemical determinations of antiglycosylating compounds and antioxidant activity from willow bark extract

Method	Amount detected
Polyphenols (gallic acid)	2.34 ± 0.62 mg/mL
Flavonoids (rutin)	0.14 ± 0.03 mg/mL
Salicylic compounds (salicin)	0.011 ± 0.005 mg/mL
Antioxidant activity (Trolox)	16.92 ± 2.16 mmol/mL

- The potential antiglycosylating effect of willow bark extract is due to significant concentrations of polyphenols and flavonoid compounds which also provide a high antioxidant activity, salicylates also having the role of maintaining oxidative stress at a low level that does not disrupt the physiological processes at the cellular level.

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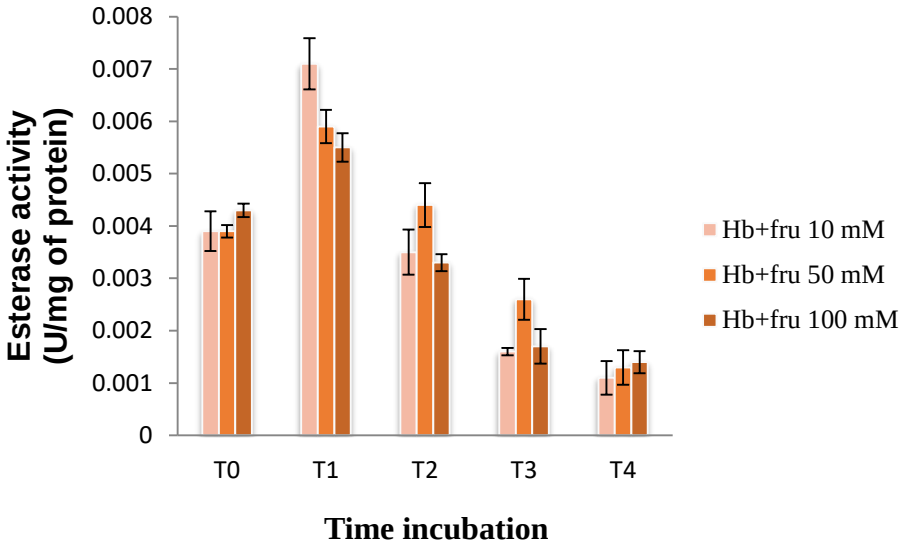

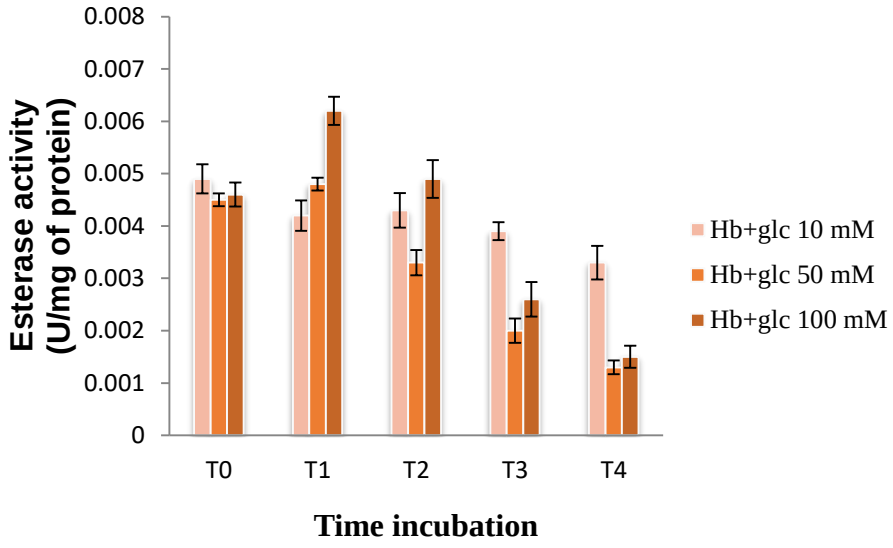

- Firstly, human erythrocytes isolated from whole peripheral blood were incubated with different concentrations of fructose/glucose (10, 50, 100 mM) and *S. alba* extract for 5 (T1), 7 (T2), 10 (T3) and 14 (T4) days.

- Both the esterase and the peroxidase activity underwent changes over time, following incubation with different concentrations of fructose and glucose, respectively.

- The binding of the first glucose/fructose molecules induced an increase in the esterase activity of hemoglobin, but subsequently the active catalytic center of the enzyme is altered, causing a decrease in enzymatic activity.

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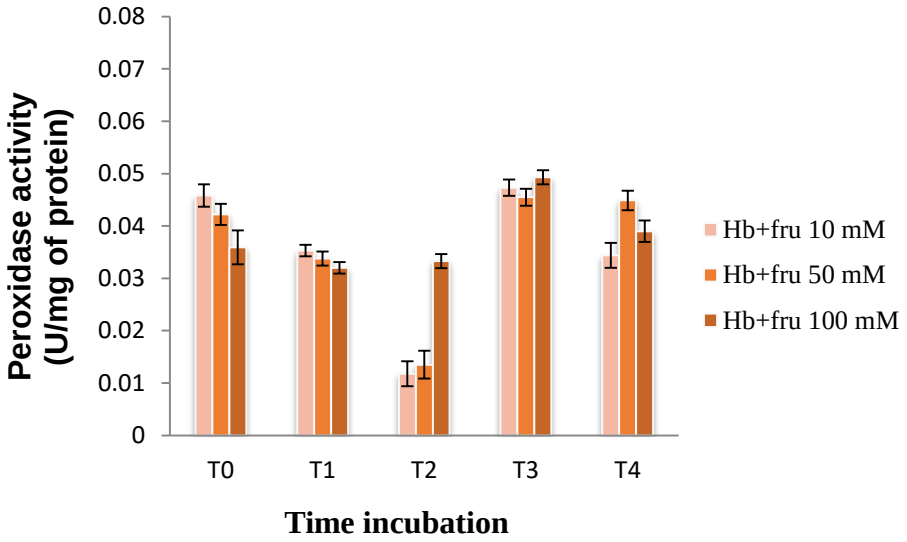

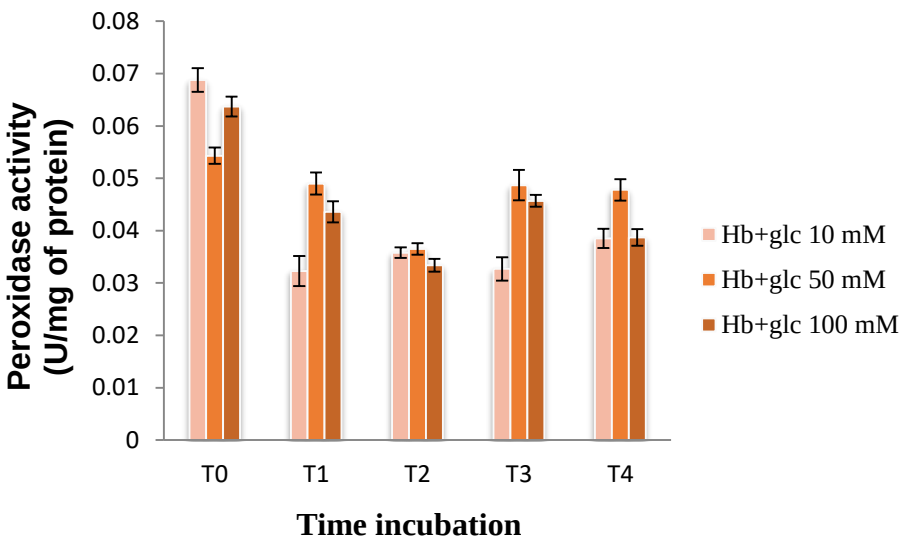

- An interesting tendency was observed in the samples incubated with different concentrations of fructose. At first, the binding of fructose molecules determined a decrease in the peroxidase activity of hemoglobin, but after 10 days there was a significant increase in enzymatic activity. This phenomenon could mean that after all the hemoglobin binding sites are occupied with fructose molecules, the fructose that remains free and its products have the role of protecting the protein against the oxidative environment, and the increase in peroxidase activity may be the result of an attempt by hemoglobin to reduce oxidative stress.

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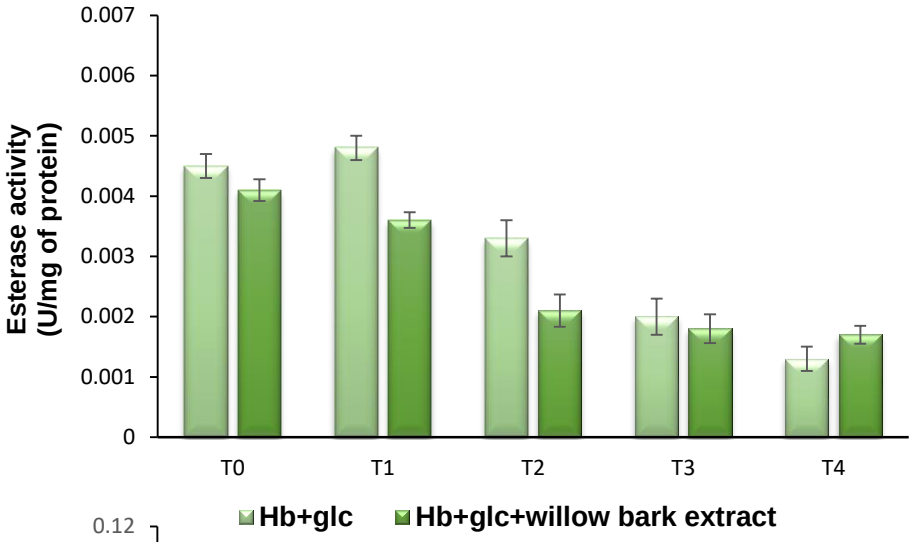

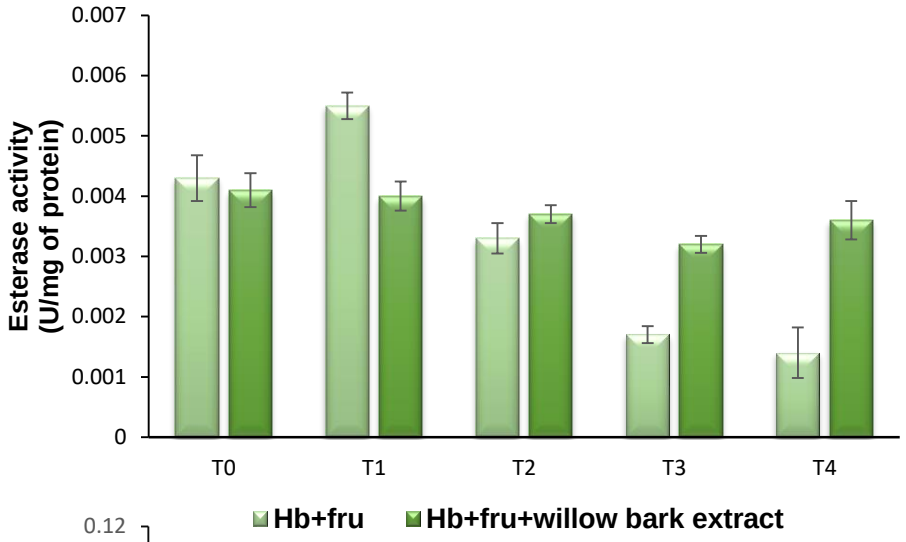

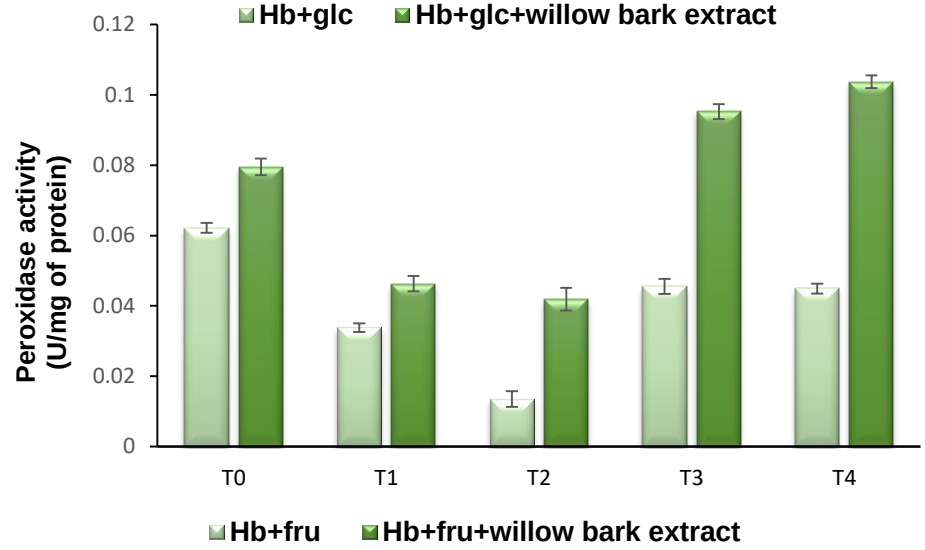

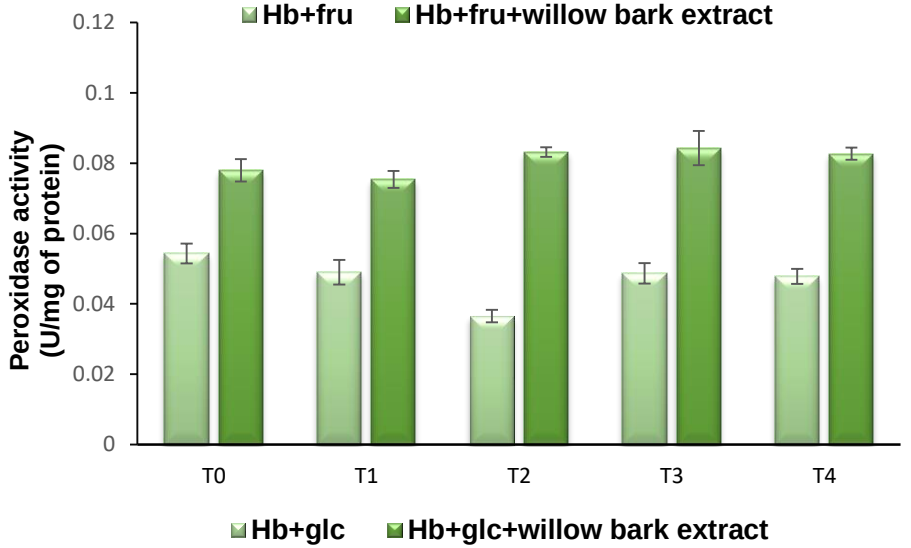

- 50 mM glucose and 100 mM fructose recorded the highest level of reproducibility, not determining sudden and unexplained variations; therefore, the influence of some natural aspirin-like compounds from Salix alba extract on changes in the enzymatic activity of hemoglobin induced by these two concentrations was analyzed.

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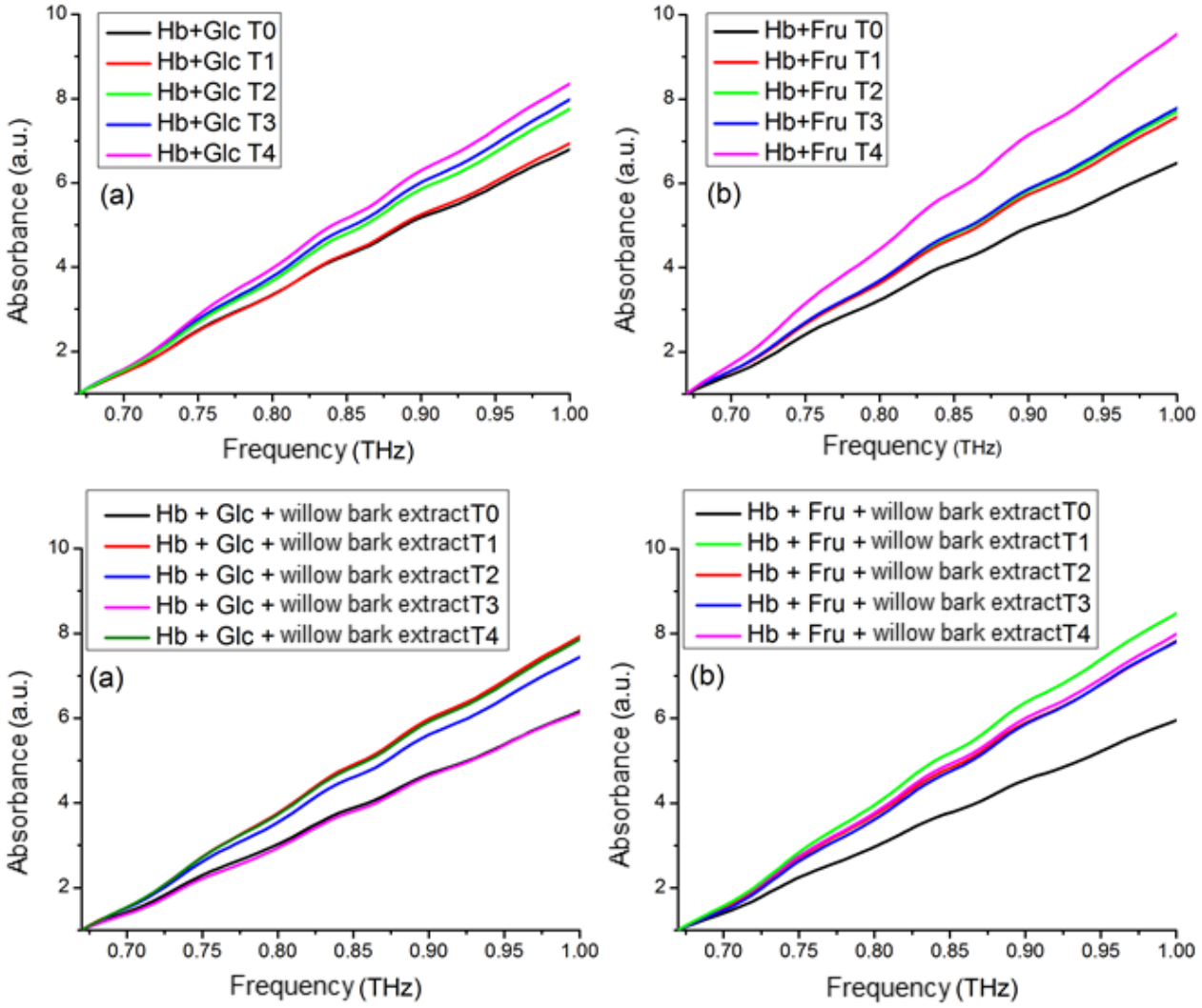
THz spectroscopy measurements



- The results obtained from the THz spectra confirmed that fructose was more reactive than glucose, so the glycation process took place more slowly than fructation. Also, the presence of *S. alba* extract showed an antiglycosylating effect, but not a total inhibition of the glycation process.

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CONCLUSIONS

- ✓ Our study showed that willow bark extract has positively influenced the changes suffered by the peroxidase activity of hemoglobin, maintaining the initial value in the case of the glycosylation process and significantly attenuating its decrease after fructosylation.

- ✓ Moreover, if *Salix alba* extract had a beneficial effect on the changes in peroxidase activity generated by both glucose and fructose binding, in the case of esterase activity it was observed that they had a significant antiglycosylating potential only in the case of fructose.

- ✓ Therefore, the salicylic compounds present in the bark extract of S. alba can be successfully used as natural substituents of aspirin to prevent glycosylation processes and AGE formation.

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