Assessment of the hypoglycemic effect of Bixin in Alloxan-induced Diabetic Rats: *in vivo* and *in silico* studies

Hady Keita[a,b], Cleydson Breno Rodrigues dos Santos[a,c]*, Matheus Mercês Ramos[d], Elias Carvalho Padilha[e], Rodolfo Bortolozo Serafim[f], Andres Navarrete Castro[g], Jesus Rafael Rodriguez Amado[a], Gabriel Monteiro da Silva[h], Irlon Maciel Ferreira[d], Silvana Giuliatti[h], and José Carlos Tavares Carvalho[a,c,d]*

[a]*Laboratory of Drugs Discovery, Department of Biological Sciences and Health. Federal University of Amapá, 68902-280, Macapá-AP, Brazil.*

[b]*Division of Post-Grade, University of the Sierra, Calle Guillermo Rojas Mijangos S/N, Esq. Av. Universidad Col. Ciudad Universitaria, Miahuatlán de Porfirio Díaz, Oax., México C.P. 70800*

[c]*Laboratory of Modeling and Computational Chemistry, Department of Biological Sciences and Health, Federal University of Amapa, 68902-280, Macapá-AP, Brazil.*

[d]*Research Group Biocatalysis and Apllied Organic Synthesis, Federal University of Amapa, 68902-280, Macapá-AP, Brazil.*

[e]*Department of Natural Active Principles and Toxicology, Faculty of Pharmaceutical Sciences, São Paulo State* University*, Araraquara, Rodovia Araraquara-Jaú Km 1, 14801-902, Araraquara-SP, Brazil.*

[f]*Department of Cellular and Molecular Biology, Ribeirão Preto Medical School, University of São Paulo (USP), Avenida Bandeirantes, 3900, 14049-900, Ribeirão Preto, Brazil.*

[g]*Laboratory of Pharmacology of Natural Products, Department of Pharmacy, Faculty of Chemistry, Universidad Autonoma Nacional de Mexico, Ciudad de Mexico, Mexico.*

[h]*Bioinformatics Group, Department of Genetics, Ribeirão Preto Medical School, University of São Paulo (USP), Avenida Bandeirantes, 3900, 14049-900, Ribeirão Preto, Brazil.*

SUPPLEMENTARY MATERIAL

Isolation of bixin from seed and characterization

The isolated compound, bixin, showed the presence of the band closer to 1600 cm^{-1}, that is a result of the asymmetric axial deformation of conjugated alkenes, of the C = O absorption band of α, β-unsaturated aliphatic esters, which occurred in 1714 cm^{-1}. Also, the C—C(C=O)—C absorption band of the α, β-unsaturated esters region, in the region of 1300-1160 cm^{-1} and the absorption band in 3171 cm^{-1}, which corresponds to the -OH group of the carboxylic acid, helped on molecular characterization too (Figure 1S), these data were compatible with those reported in the literature for the bixin substance (Jondiko et al., 1989; Sousa et al., 1991; Costa & Chaves, 2005; Silverstein et al., 2007).

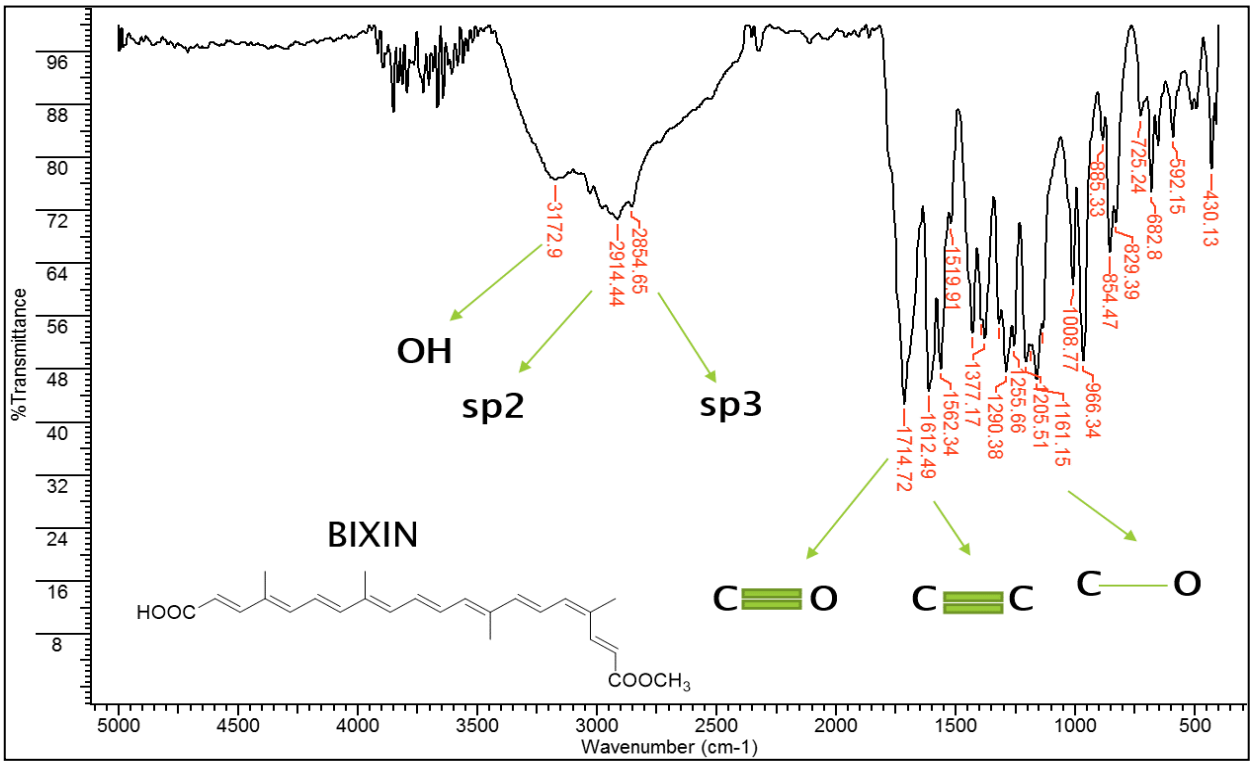

Figure 1S. Infrared spectrum corresponding to the bixin.

The ^{13}C NMR spectroscopy analysis corroborates with the elucidation of the bixin structure. The four signals that are going since 12 to 20 ppm correspond to

methyls, the peak on 51 correspond to C-O groups, the peaks that are going since 115 to 151ppm correspond to unsaturated 18 carbons, and the peaks on 168 and 171ppm correspond to C=O groups (Figure 2S), these data were compatible with those reported in the literature for the bixin substance (Jondiko et al., 1989; Sousa et al., 1991; Costa & Chaves, 2005; Silverstein et al., 2007).

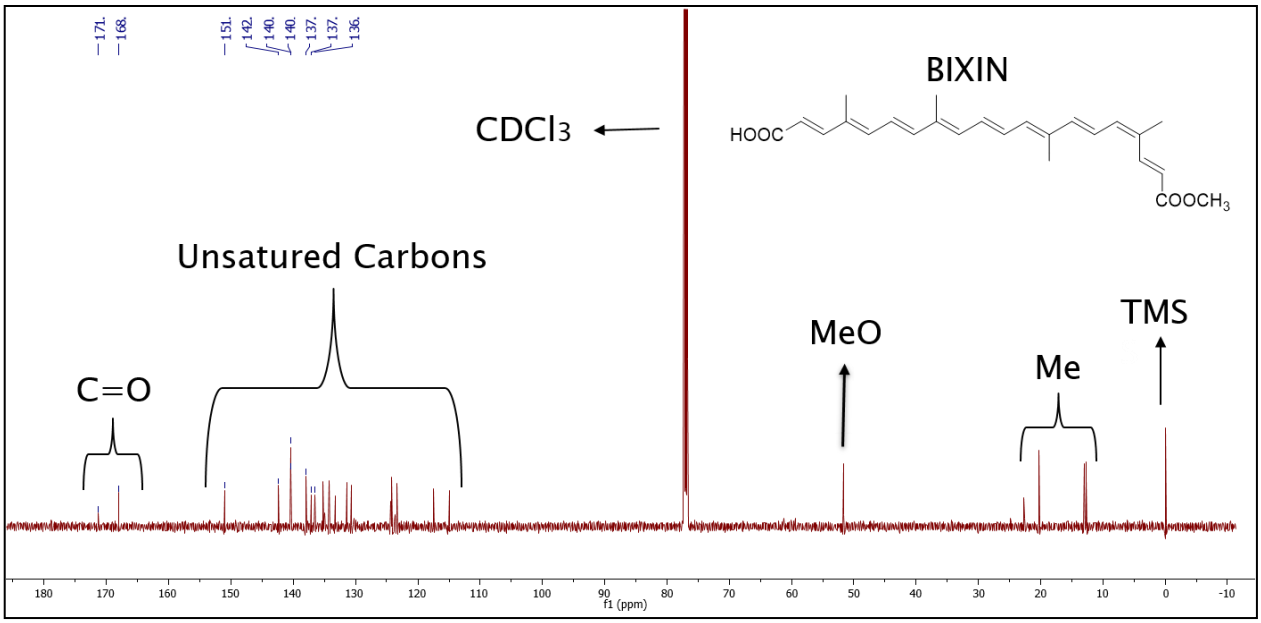

Figure 2S. ^{13}C-NMR spectrum ($CDCl_3$) corresponding to the bixin.

It is possible to observe in the Figure 1S, the presence of the band closer to 1600 cm^{-1}, that is result of the asymmetric axial deformation of conjugated alkenes, of the C = O absorption band of α,β-unsaturated aliphatic esters, which occurred in 1714 cm^{-1}. Also, the C—C(C=O)—C absorption band of the α,β-unsaturated esters region, in the region of 1300-1160 cm^{-1} and the absorption band in 3171 cm^{-1}, which corresponds to the -OH group of the carboxylic acid.

It is possible to observe in the Figure 2S, the four signals that are going since 12 to 20 ppm correspond to methyls, the peak on 51 correspond to C-O groups, the peaks that are going since 115 to 151ppm correspond to unsaturated 18 carbons, and the peaks on 168 and 171ppm correspond to C=O groups.

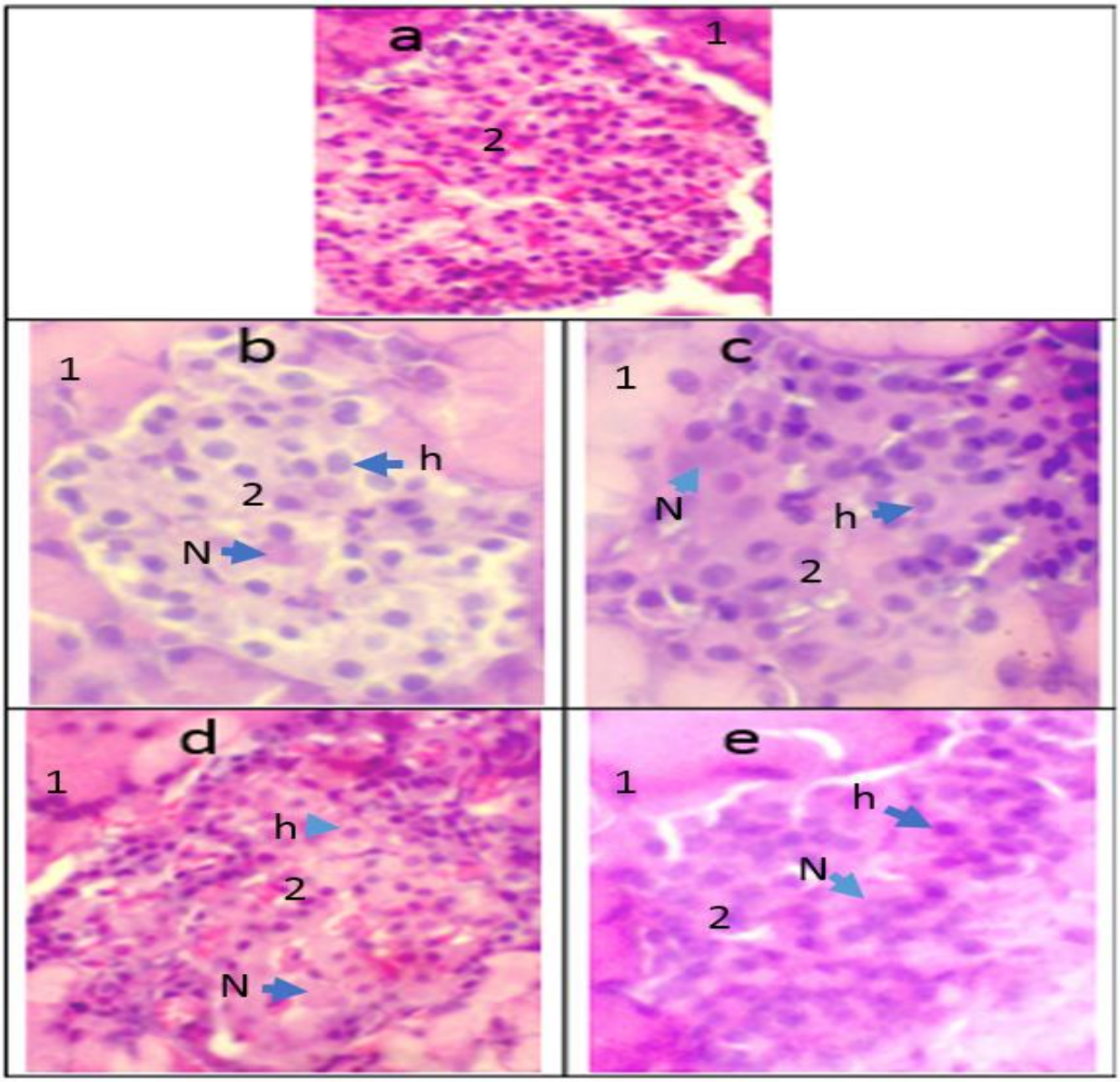

Figure 3S. Histopathological changes in the rat pancreas treated orally with bixin (10 mg/kg) and metformin (100 mg/kg). H&E staining of rat pancreas (× 400). **a**-Control group displays normal size of islets of Langerhans and normal cells density; **b**-Bixin, **c**-metformin display nuclear hypertrophy of islets of Langerhans cells, **d**-Diabetes and e-tween display marked destruction of islets of Langerhans cells and apparent decrease in cell density of islets of Langerhans. **1**: exocrine cells. **2**: endocrine cells, **h**: hypertrophy of nuclear cells, **N**: Necrotic cell. Arrowhead displays alteration.

References:

Costa, Charllyton Luis S. da, & Chaves, Mariana H.. (2005). Extração de pigmentos das sementes de Bixa orellana L.: uma alternativa para disciplinas experimentais de química orgânica. Química Nova, 28(1), 149-152. https://dx.doi.org/10.1590/S0100-40422005000100026

Silverstein, R.M.; Webster, F.X.; Kiemle, D.J. Identificação espectrométrica de compostos orgânicos. 7ª Ed. Rio de Janeiro: LTC, 2007. Número de Chamada: 543.42 S587i 7.ed

Sousa, P. S.; Matos, M. E. O.; Matos, F. J. A.; Machado, M. I. L.; Craveiro, A. A.; Constituintes químicos ativos de plantas medicinais brasileiras, Ed. UFC: Fortaleza, 1991.

Jondiko, I. J.O., Pattenden, G (1989). Terpenoids and an apocarotenoid from seeds of Bixa orellana, Phytochemistry, Vol. 28 (11), 3159-3162. https://doi.org/10.1016/0031-9422(89)80298-5.