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# Anti-obesity and anti-steatotic effects of Bixin (apocarotenoid from *Bixa orellana I.* seeds): Dose-dependent correlations with bioactivity

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#### **INTRODUCTION & AIM** MetS (Metabolic syndrome) - Constellation of diseases: Diabetes, Obesity, Hypertension, Cardiovascular diseases. Hepatic complication of MetS: NAFLD 2.0 -Globally, over 1 billion people are overweight<sup>1</sup>, 25-35% suffer from NAFLD<sup>2</sup>. The global prevalence of NAFLD among the obese is 75.54% <sup>1</sup>. The present study was aimed to evaluate dose-dependent anti-obesity and anti-NAFLD (A) efficacy of bixin. **(B) Obesity NAFLD** 0.5 -(Non-alcoholic fatty Hepatocellular **NASH** liver disease) **Cirrhosis** Diabetes Metabolic syndrome **Steatosis Fat Steatosis** Late stages Inflammation in >5% of fibrosis **(B) Ballooning** hepatocytes) Fibrosis Hypertension Liver transplant or death Cardiovascular OD (490 diseases **Annatto seeds** (Bixa orellana l.) Terpenoids Nutraceuticals Bixin Apocarotenoid) Glucosinolates Polyphenols **Carotenoids METHOD** Bixin structure **Food colorant** Pure chemical Bixin Dose-dependent effects of Bixin on 3T3-L1 and HepG2 cells OA-induced steatosis model Differentiation of 3T3L1 **Mature Adipocytes Preadipocytes** (3T3-L1)Bixin treatment HepG2 cells confluence Day 0 Day 2 Day 4 Day 6 1mM Oleic acid (48 hrs) 2 Days 2 Days 2 Days 2 Days 2 Days 0.25µM Dexamethasone 2 Days 500μM 3-isobutyl-1-**Day 10** Day 8 Steatosis model methylxanthine (IBMX) **—** Bixin Treatment 10μg/ml Insulin (24 hrs) (DMI) Validation in *in-vitro* models Cell viability assay Oil Red O staining **Oxidative stress** (Lipid staining) assays **RESULTS & DISCUSSION** Dose-dependent cytotoxicity assessment by MTT assay (A) (B) 3T3-L1 Adipocytes **HepG2 cells** 100-100 ¬ **Selection of** non-toxic doses for anti-obesity

**Figure 1: Concentration-dependent effects of bixin on cell viability.** (A) cell viability in 3T3-L1 cells (B) cell viability in HepG2 cells. Values are expressed as mean ± SD of three independent experiments performed in triplicates.

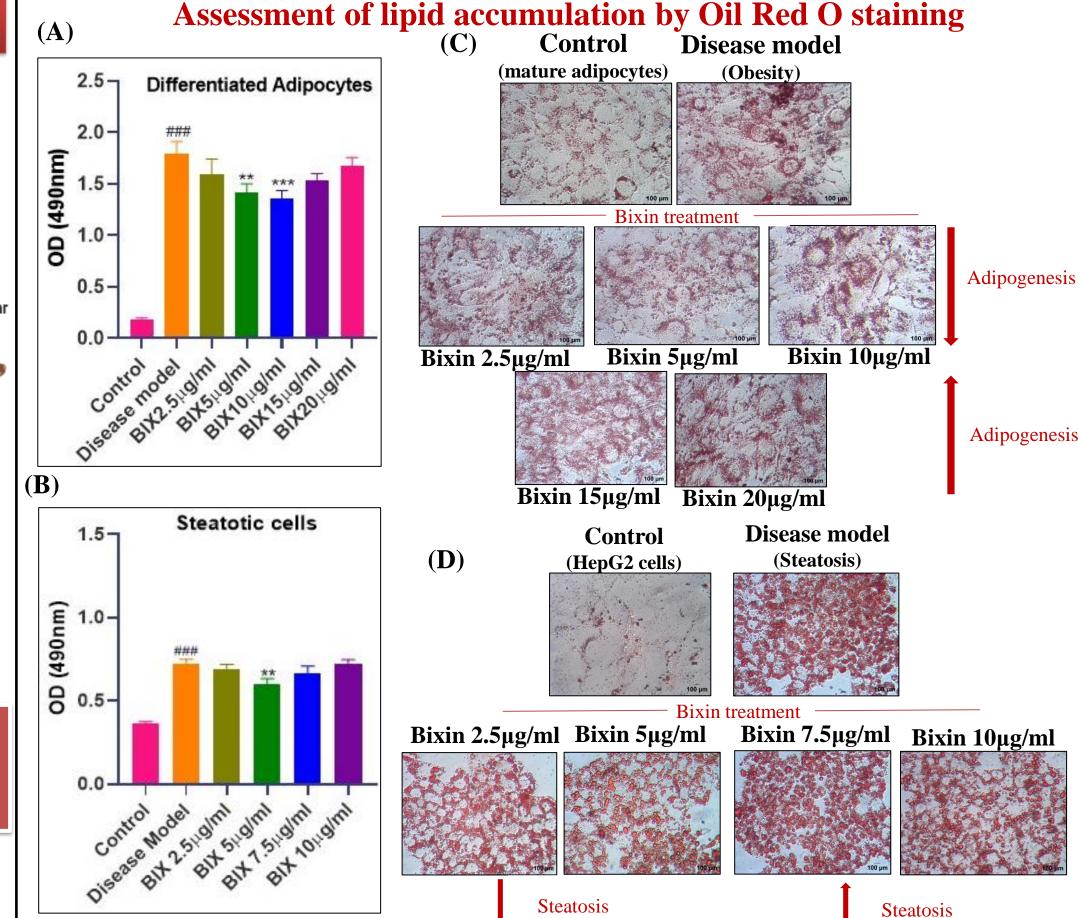
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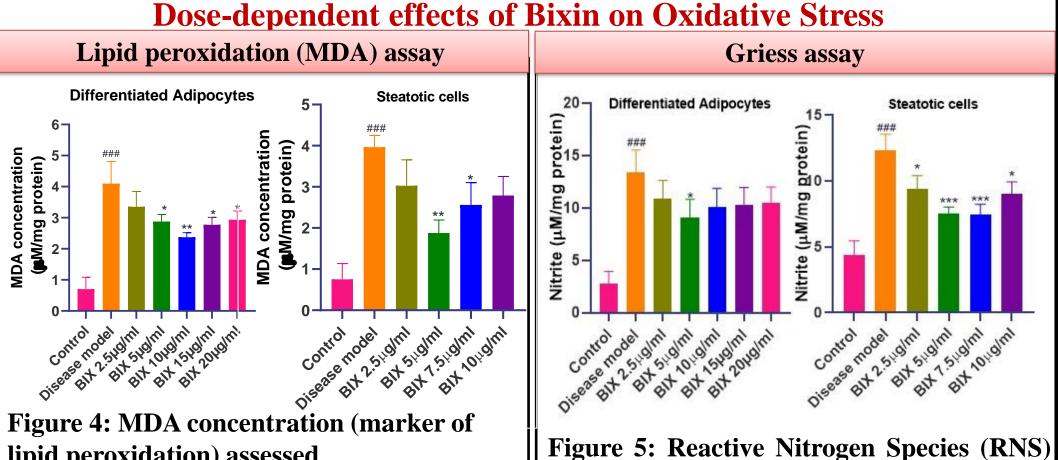
and anti-NAFLD

effect

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**Figure 3. Inhibitory effects of bixin on lipid accumulation in** *in vitro* **disease models.** (i) **(A-B)** spectrophotometric quantification of lipid accumulation (ii) **(C-D)** Representative photomicrographs of Oil Red O staining.



lipid peroxidation) assessed spectrophotometrically by TBARS assay.

DCFDA assay

levels in disease and treated groups

CONCLUSION

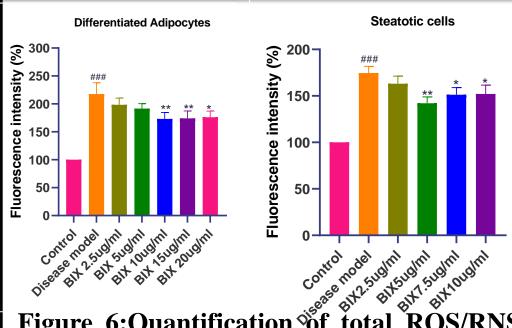


Figure 6:Quantification of total ROS/RNS levels by DCFDA assay.

Values are expressed as mean  $\pm$  S.D. of a minimum of three independent experiments. ### P < 0.001, relative to the control group. \*P < 0.05,\*\*P < 0.01, and \*\*\*P < 0.001 relative to the disease model.

## CONCLUSION

- Bioactivity of the apocarotenoid bixin was found to be **dose-dependent**.
- Bixin exhibited anti-obesity and anti-NAFLD effects at 10μg/ml and 5μg/ml respectively.
- However, at higher concentrations, that is, above 10μg/ml in differentiated adipocytes and 5μg/ml in steatotic cells, it ceased to inhibit lipid accumulation. This adipogenic/steatotic response was accompanied by elevated oxidative stress/lipid peroxidation, indicating a pro-oxidant effect at higher doses, which implicates its anticancer potential.
- Evaluation of dose-dependent pharmacological efficacy of natural products is emphasized.

### REFERENCES

- 1. World Health Organization (WHO). Obesity and Overweight. 2021; <a href="https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight">https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight</a>.
- . Le, Michael H., et al. "2019 Global NAFLD prevalence: a systematic review and meta-analysis." Clinical Gastroenterology and Hepatology 20.12 (2022): 2809-2817.
- 8. Puri, Sonakshi, et al. "Comparative bioactivity assessment of bixin pigment and associated phytochemicals extracted from annatto seeds using conventional and green solvents." Journal of Food and Drug Analysis 32.2 (2024): 168.
- Müller, Fabrice A., and Shana J. Sturla. "Human in vitro models of nonalcoholic fatty liver disease." Current Opinion in Toxicology 16 (2019): 9-16.
- 5. Takahashi, Nobuyuki, et al. "Bixin regulates mRNA expression involved in adipogenesis and enhances insulin sensitivity in 3T3-L1 adipocytes through PPARγ activation." Biochemical and biophysical research communications 390.4 (2009): 1372-1376.