

Supplementary File for Review Only

Apigeninidin-enrich *Sorghum bicolor* (L. Moench) extracts alleviate Aflatoxin B₁-induced dysregulation of male rat's hypothalamic-reproductive axis.

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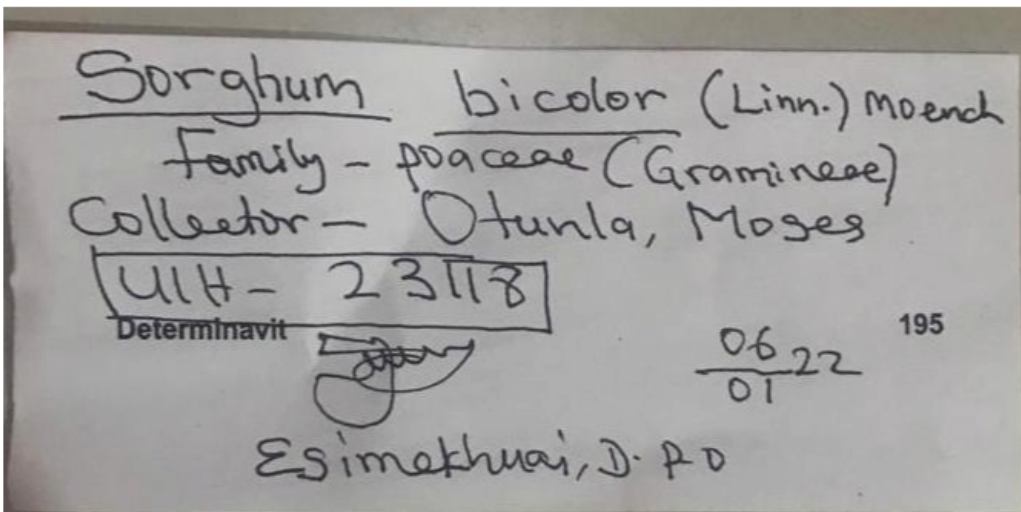
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Running Title: Aflatoxin B₁ toxicity and apigeninidin in exposed rats
Collection, identification, extraction and characterization of Apigeninidin from *Sorghum bicolor* by Liquid Chromatography-Mass Spectrophotometry LC-MS.

Dried *Sorghum bicolor* sheets (5 Kg) was purchased from Bodija Market, Ibadan, Nigeria and were pulverized into very fine powder. Geographically, Bodija market lies between longitude 3 54'36''E and 3 55' 12''E and latitude 7 25' 52''N and 7 26' 22''N. The plant samples were transported in a polythene bag to the Herbarium of the Department of Botany, University of Ibadan, Ibadan, Nigeria for identification by a veteran taxonomist Mr. Donatus Esimekhuai. The sample of *S. bicolor* were identified and deposited in the Department of Botany and a voucher

specimen -Accession number: UIH-23118- assigned for future reference.



Experimentation with - *S. bicolor*- complied with all relevant institutional, national, and international guidelines and legislation. The plant was sorted to remove dirt and other extraneous materials and pulverized into fine powder.

Extraction and phytochemical characterization of *S. bicolor*

Sample of pulverized *S. bicolor* (120 g) is degreased with CH_2Cl_2 and subsequently extracted with CH_2Cl_2 : MeOH 10:1 (twice) at 60°C and 0.1% HCl in EtOH at room temperature. Each filtrate was evaporated off to give SBE-05 (2.6 g), SBE-06 (1.44 g) and SBE-07 (4.85g) as brownish-red solid.

LC-MS analysis

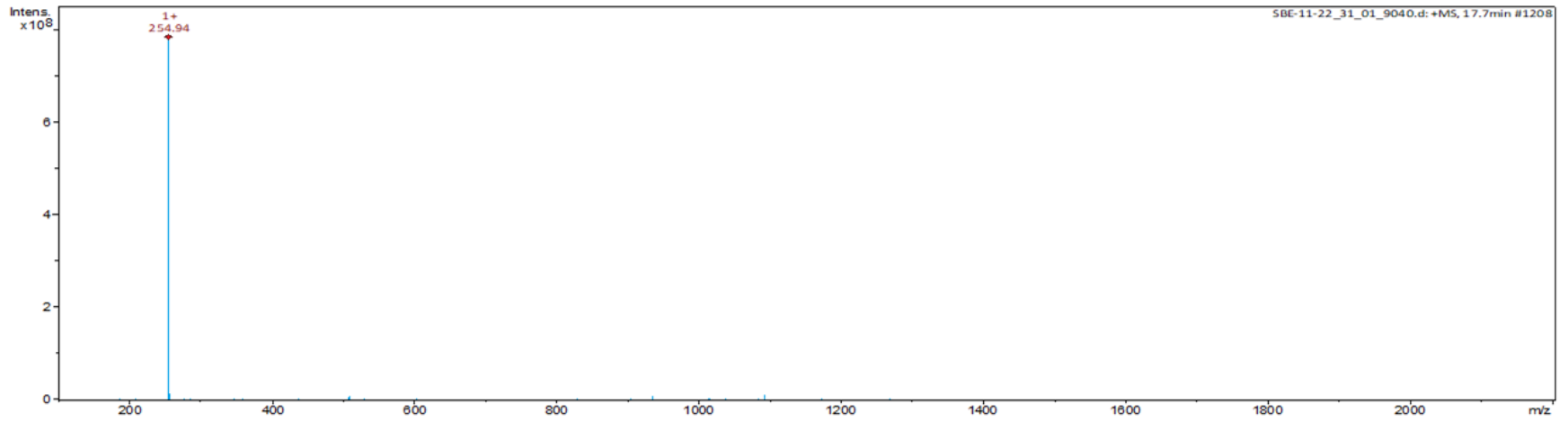
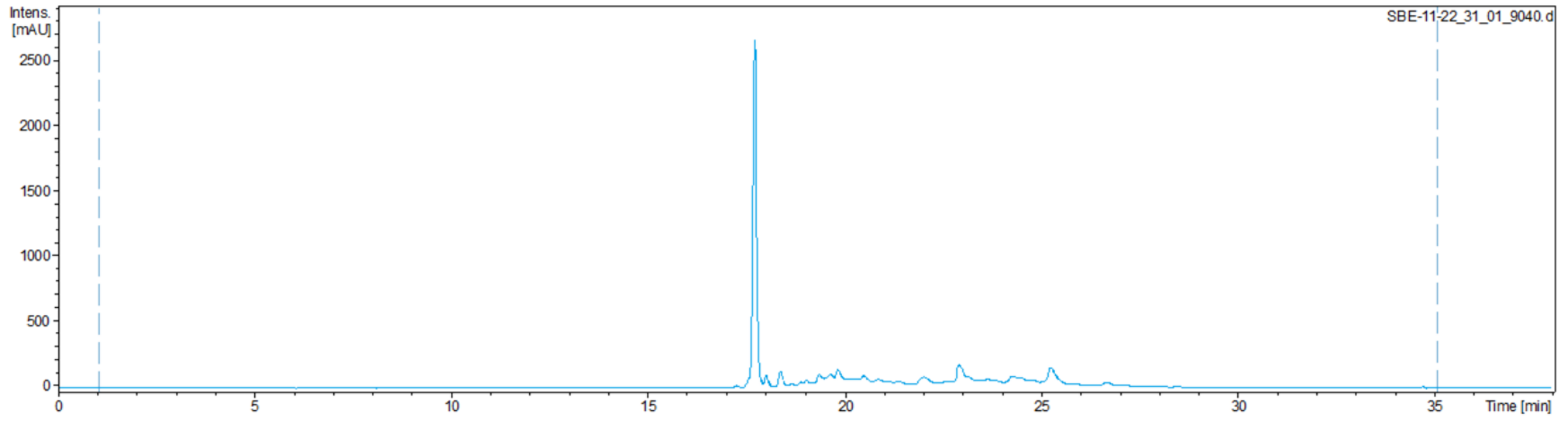
The isolated solid from each fraction – SBE-05, SBE-06 and SBE-07 – was analyzed were analyzed by LC-MS, monitoring at 420 nm, on a Bruker amaZon SL ion trap mass spectrometer coupled to an Agilent 1260 HPLC. Chromatography was performed on Phenomenex C18 reversed phase HPLC column (250 × 4.6 mm; S/NO: H17-238591) at a flow rate of 0.5 mL/min;

using 0.1% v/v formic acid in H₂O (solvent A) and 0.1% v/v formic acid in MeCN (solvent B).

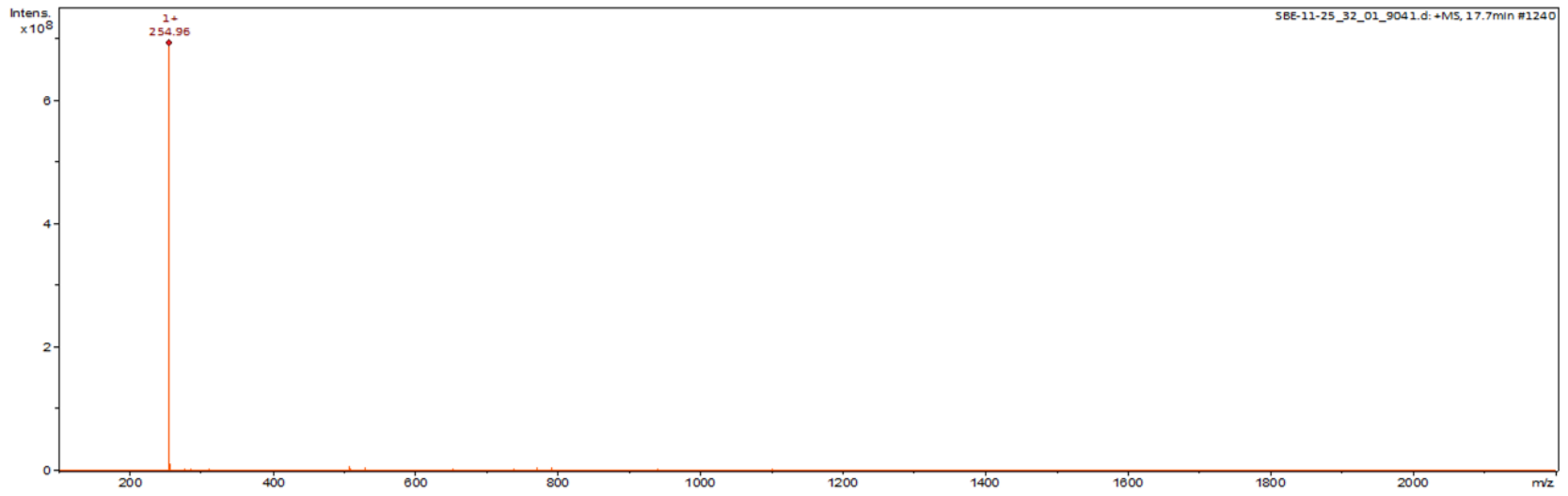
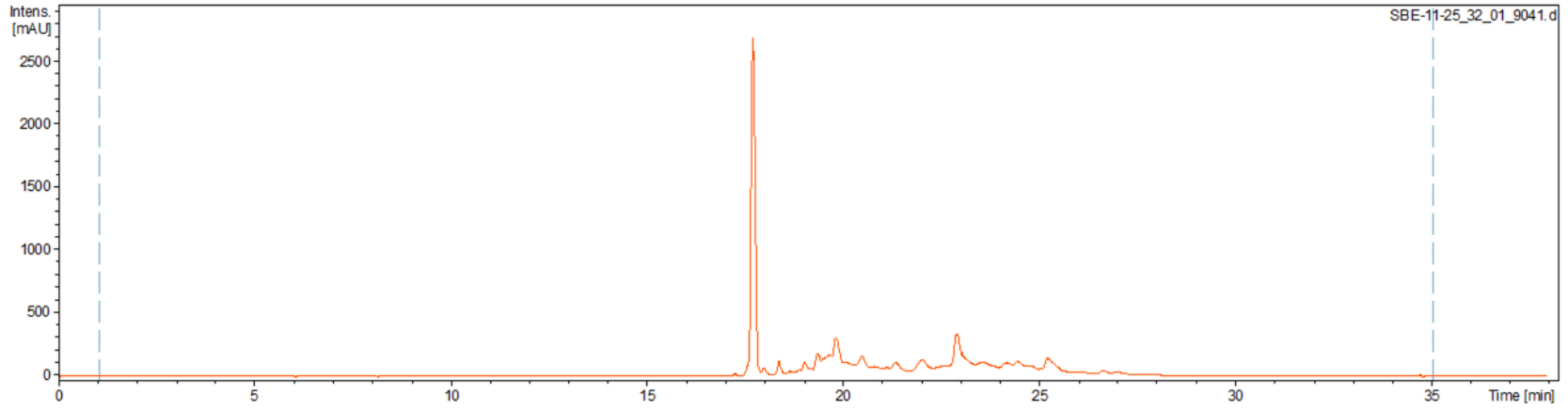
The solvent gradient for chromatography elution is as follows: 0–5 min at 5% solvent B; 5–25 min from 5% to 100% solvent B; 25–28 min at 100% solvent B; 28–29 min from 100% to 5% solvent B; and 29–32 min at 5% solvent B. Mass spectrometry data were collected in the positive and negative ionization modes in the mass range m/z 100–1000 Da.

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SBE-05

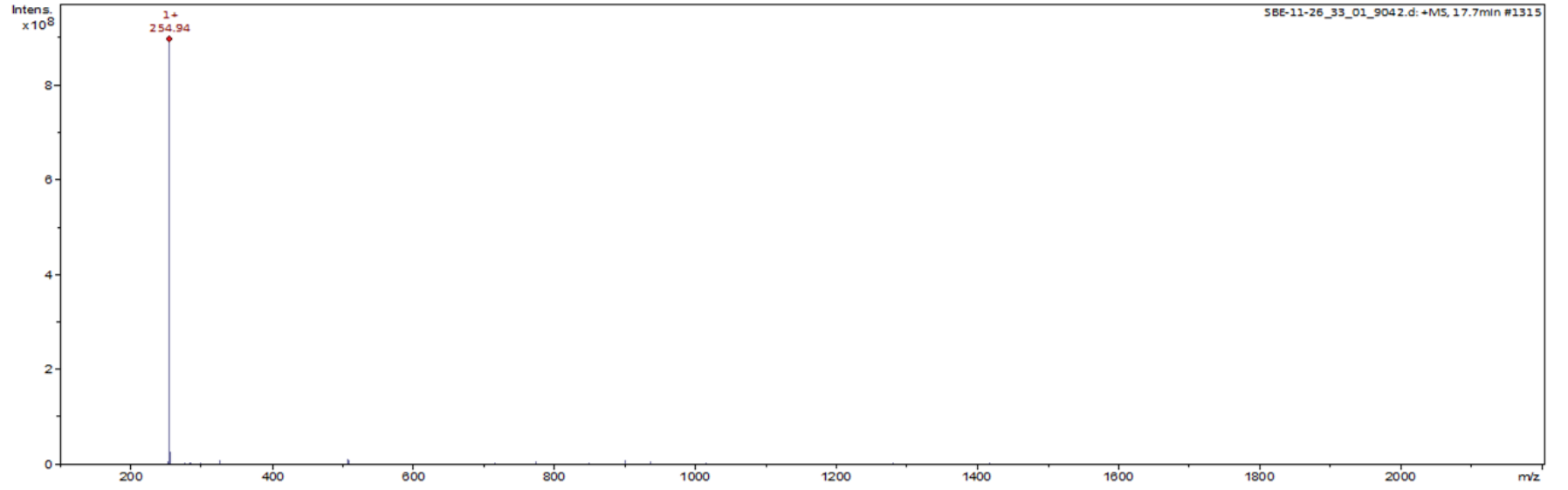
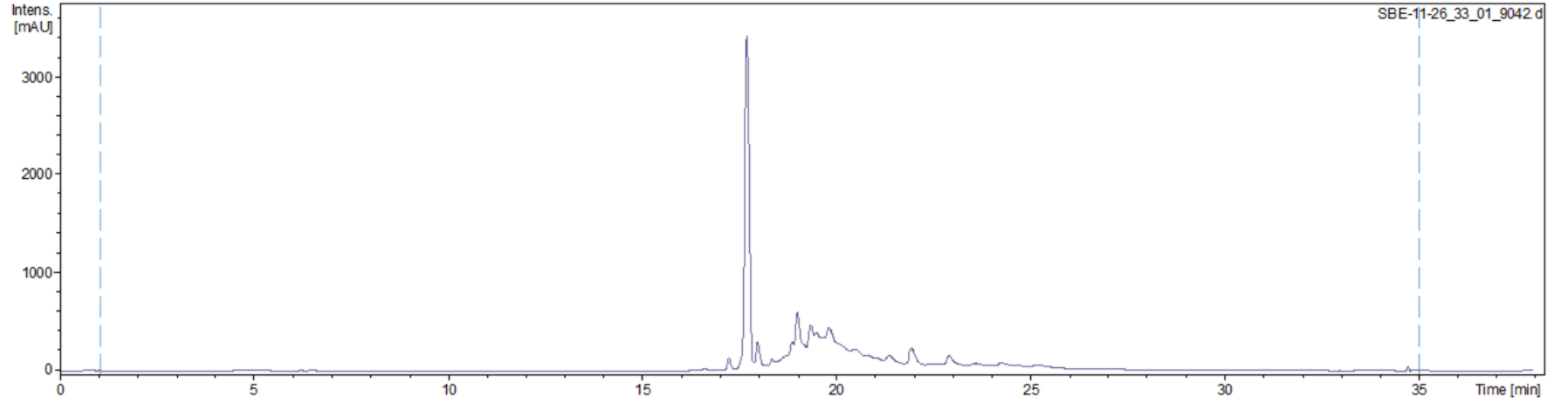


SBE-06

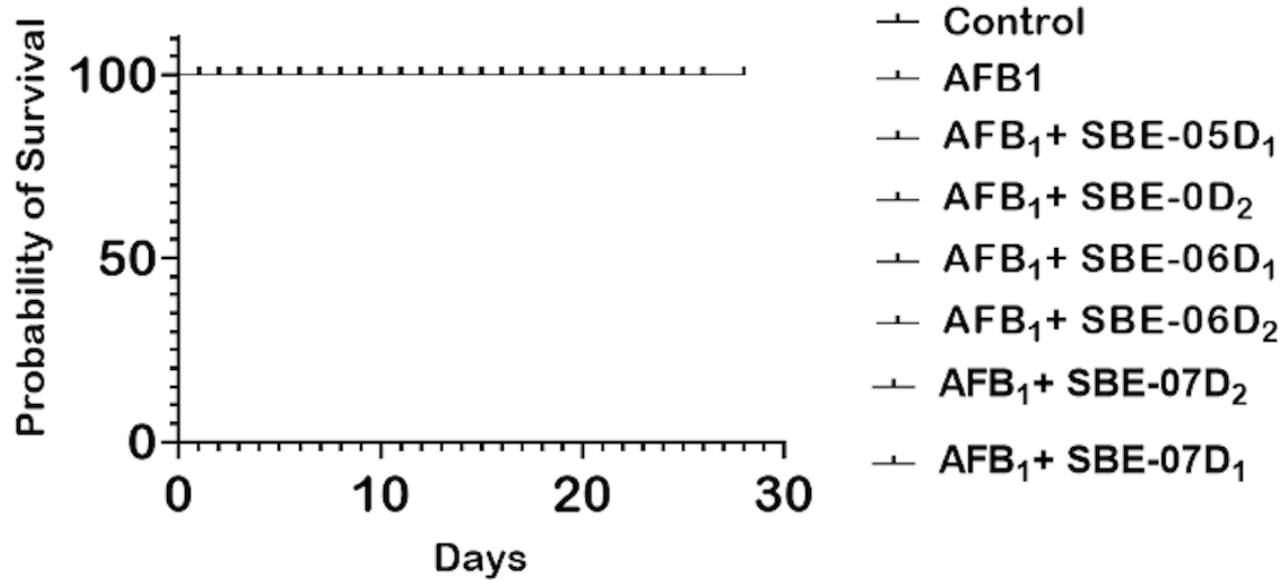


(iii)

SBE-07

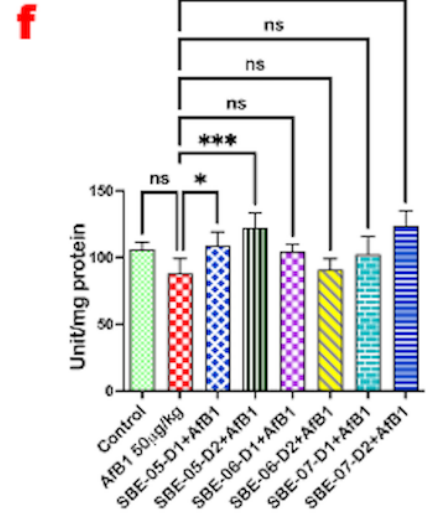
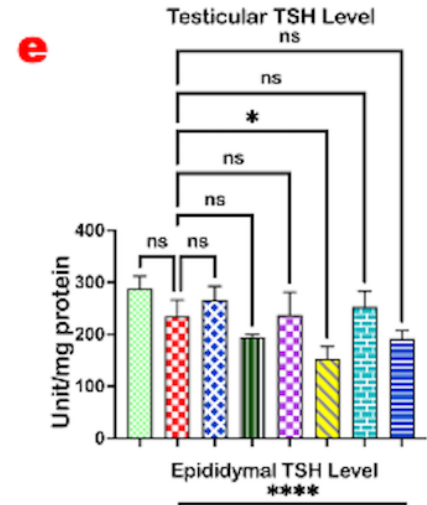
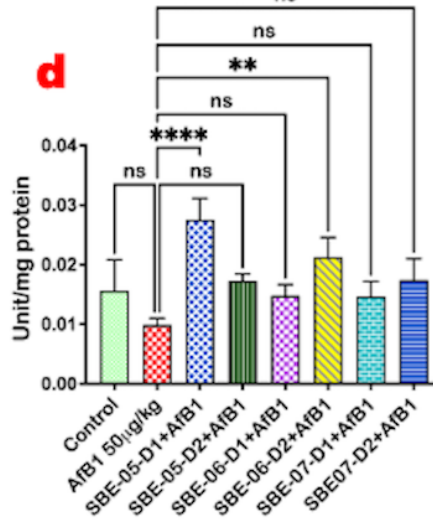
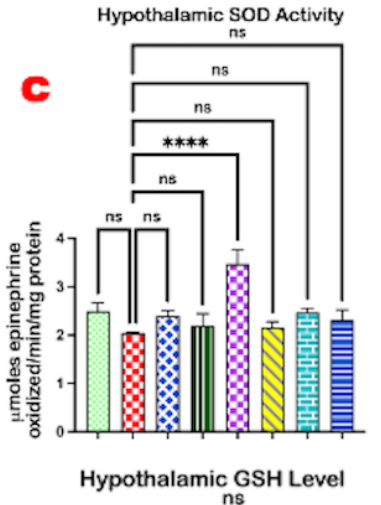
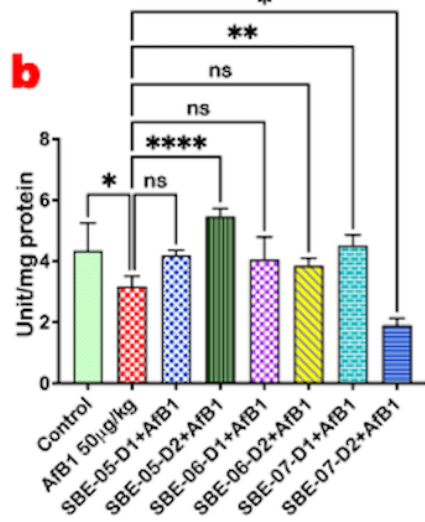
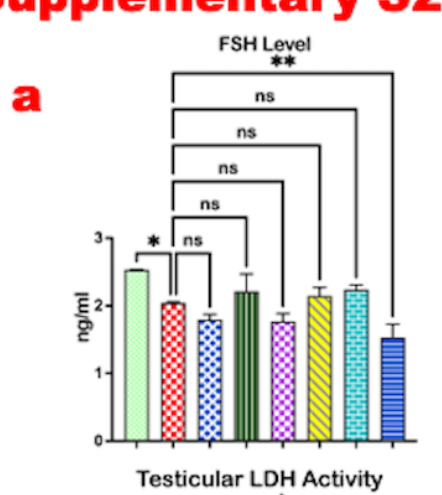


Supplementary S1



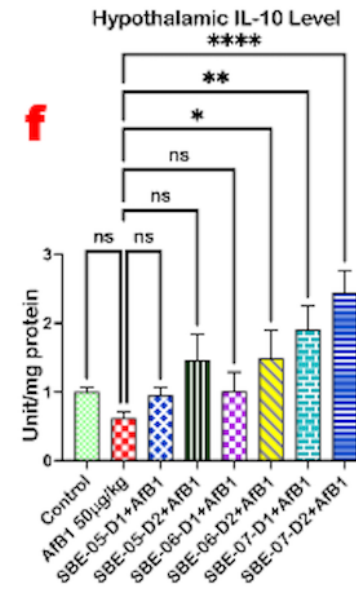
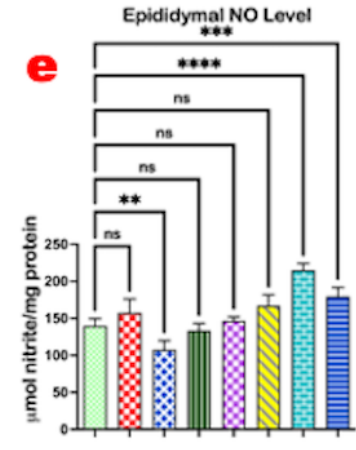
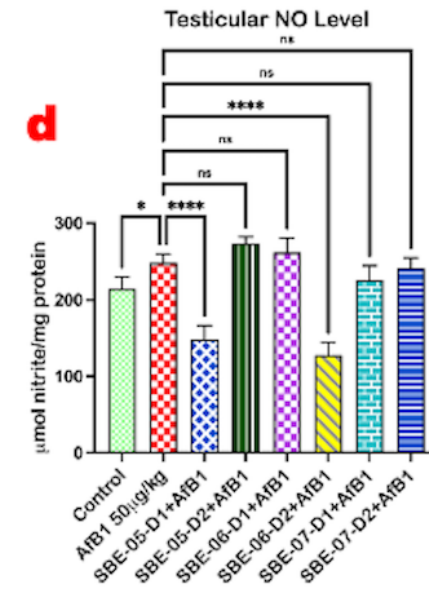
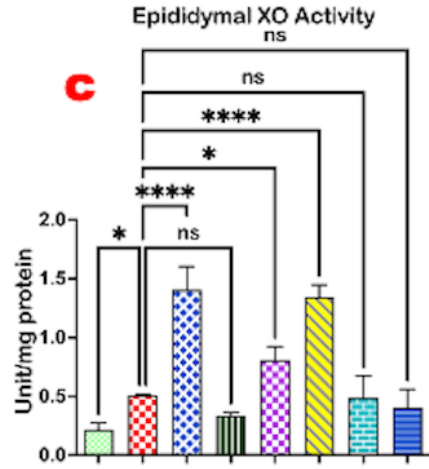
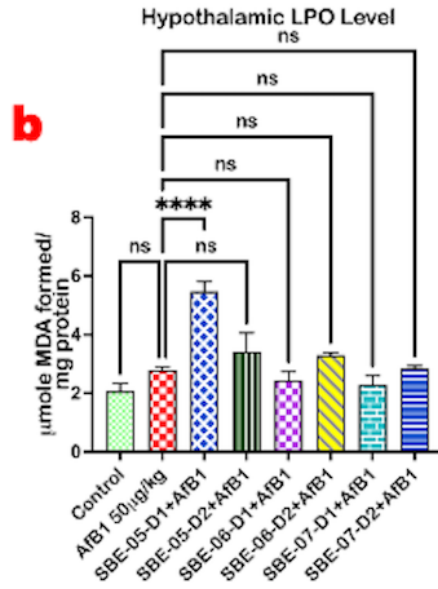
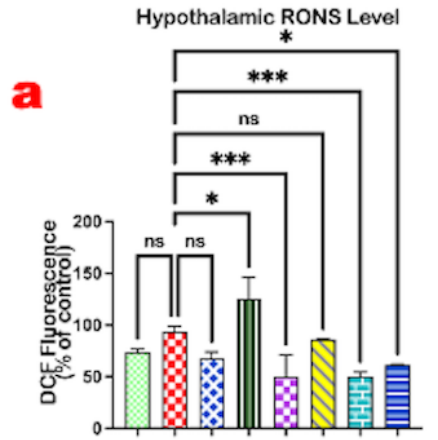
Supplementary Figure 1: Effect of API and AFB₁ treatment on experimental rats -Kaplan-Meyers Survival Indicator. AFB₁, 50µg/kg; AFB₁+SBE-05-D1, (0.05+5) mg/kg; AFB₁+SBE-5-D2, (0.05+10) mg/kg; AFB₁+SBE-06-D1, (0.05+5) mg/kg; AFB₁+SBE-06-D2, (0.05+10) mg/kg; AFB₁+SBE-07-D1, (0.05+5) mg/kg; AFB₁+SBE-07-D2, (0.05+10) mg/kg.

Supplementary S2



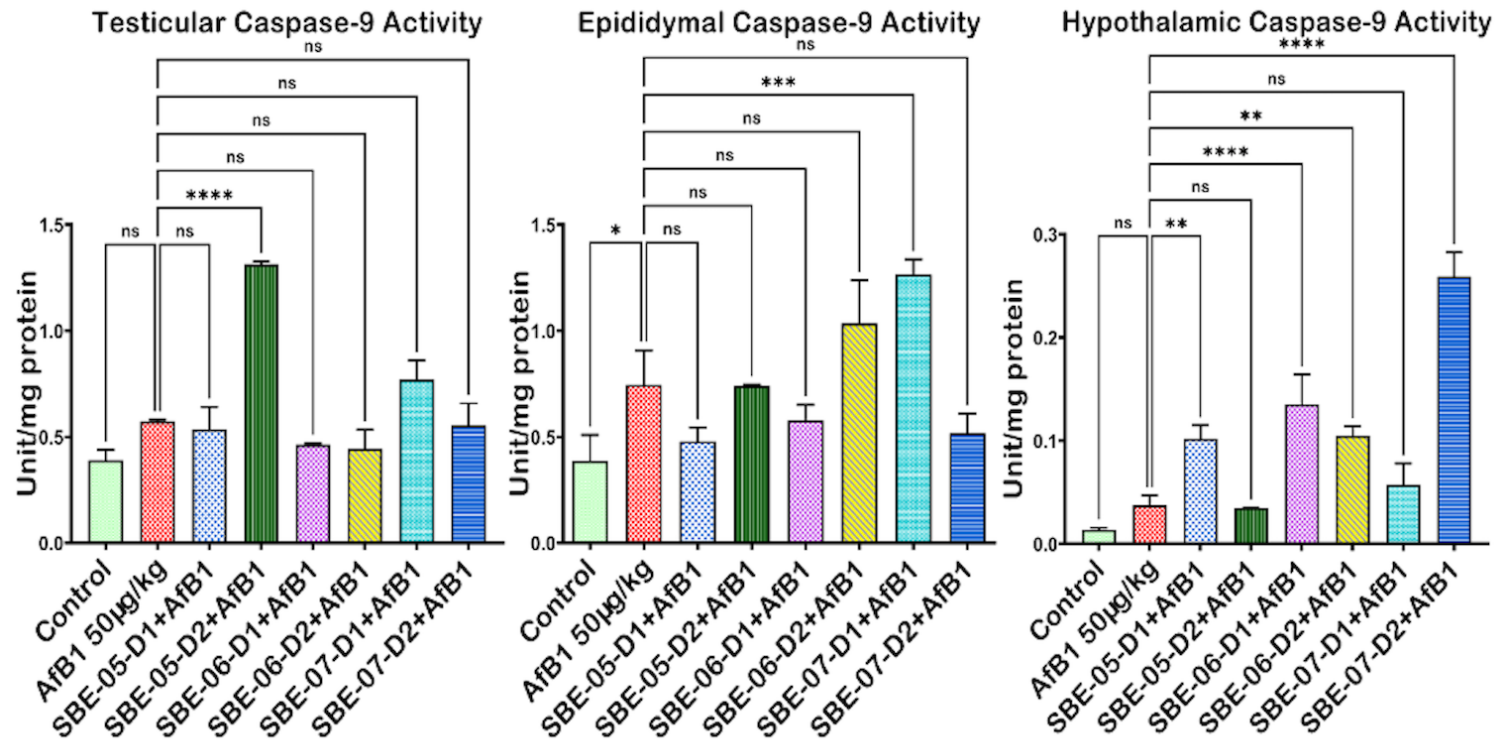
Supplementary Figure 2: Effect of API on testicular activities of FSH, LDH, and the levels of testicular, epididymal and hypothalamic SOD, GSH and TSH in AFB₁-treated rats. AFB₁, 50µg/kg; AFB₁+SBE-05-D1, (0.05+5) mg/kg; AFB₁+SBE-5-D2, (0.05+10) mg/kg; AFB₁+SBE-06-D1, (0.05+5) mg/kg; AFB₁+SBE-06-D2, (0.05+10) mg/kg; AFB₁+SBE-07-D1, (0.05+5) mg/kg; AFB₁+SBE-07-D2, (0.05+10) mg/kg. Values are expressed as mean (SD) for 6 rats per group. *: Values differ significantly from control ($p<0.05$). **: Values differ significantly from AFB₁ alone ($p<0.05$). ns: not significant. FSH: follicle stimulating hormone; LDH: lactate dehydrogenase; SOD: superoxide dismutase; GSH: glutathione and TSH: total thiol.

Supplementary S3



Supplementary Figure 3: Effect of API on the levels of testicular, epididymal and hypothalamic RONS, LPO, XO, NO, IL-and 10 in AFB₁-treated rats. AFB₁, 50µg/kg; AFB₁+SBE-05-D1, (0.05+5) mg/kg; AFB₁+SBE-5-D2, (0.05+10) mg/kg; AFB₁+SBE-06-D1, (0.05+5) mg/kg; AFB₁+SBE-06-D2, (0.05+10) mg/kg; AFB₁+SBE-07-D1, (0.05+5) mg/kg; AFB₁+SBE-07-D2, (0.05+10) mg/kg. Values are expressed as mean (SD) for 6 rats per group. *: Values differ significantly from control ($p<0.05$). **: Values differ significantly from AFB₁ alone ($p<0.05$). ns: not significant. RONS: reactive oxygen and nitrogen species; LPO: lipid peroxidation; XO: xanthine oxidase; NO: nitric oxide; IL-10: Interleukine-10.

Supplementary S4



Supplementary Figure 4: Effect of API on the activity of Caspase-9 in the testes, epididymis and hypothalamus of AFB₁-exposed rats. AFB₁, 50µg/kg; AFB₁+SBE-05-D1, (0.05+5) mg/kg; AFB₁+SBE-5-D2, (0.05+10) mg/kg; AFB₁+SBE-06-D1, (0.05+5) mg/kg; AFB₁+SBE-06-D2, (0.05+10) mg/kg; AFB₁+SBE-07-D1, (0.05+5) mg/kg; AFB₁+SBE-07-D2, (0.05+10) mg/kg. Values are expressed as mean (SD) for 6 rats per group. *: Values differ significantly from control ($p < 0.05$). **: Values differ significantly from AFB₁ alone ($p < 0.05$). ns: not significant.