

## *Supplementary Material*

### Supplementary Tables

**Supplementary Table 1** The step gradient and isocratic solvent composition for separation of EBN sample

Time (min)	Acetonitrile	0.1% Formic acid in Water
0	5.0	95.0
5	5.0	95.0
20	95.0	5.0
25	95.0	5.0
25	5.0	95.0
30	5.0	95.0

**Supplementary Table 2** Identification of the major EBN chromatogram peak from LC-MS analysis

Peak	Rt (min)	m/z	Molecular weight (g/mol)	Molecular formula	Probable compound
1	1.51	118.000	117.15	C <sub>5</sub> H <sub>11</sub> NO <sub>2</sub>	L-Valine
2	1.76	160.842	161.27	C <sub>6</sub> H <sub>15</sub> NO <sub>2</sub> Si	Trimethylsilyl l-Alanine
3	11.53	371.218	372.5	C <sub>23</sub> H <sub>32</sub> O <sub>4</sub>	Testosterone, 3,17beta-bisAc
4	12.58	385.233	386.5	C <sub>24</sub> H <sub>34</sub> O <sub>4</sub>	Progesterone 16,17-acetonide
5	12.84	187.097	186.18	C <sub>8</sub> H <sub>9</sub> NO <sub>4</sub>	4-Pyridoxic Acid-d3
6	13.73	201.113	202.25	C <sub>9</sub> H <sub>18</sub> N <sub>2</sub> O <sub>3</sub>	L-Leucyl-L-alanine
7	14.42	173.118	174.2	C <sub>6</sub> H <sub>14</sub> N <sub>4</sub> O <sub>2</sub>	L-Arginine
8	14.64	215.128	215.13	C <sub>6</sub> H <sub>8</sub> F <sub>3</sub> NO <sub>4</sub>	(2S)-3-Hydroxy-2-methyl-2-[(2,2,2-trifluoroacetyl)amino]propanoic acid
9	15.12	225.149	225.16	C <sub>8</sub> H <sub>10</sub> F <sub>3</sub> NO <sub>3</sub>	Proline, N-(trifluoroacetyl)-, methyl ester
10	15.48	229.144	231.29	C <sub>11</sub> H <sub>21</sub> NO <sub>4</sub>	N-(Tert-Butoxycarbonyl)-L-leucine
11	15.48	229.144	231.29	C <sub>11</sub> H <sub>21</sub> NO <sub>4</sub>	N-(Tert-Butoxycarbonyl)-L-leucine
12	16.31	243.16	243.21	C <sub>10</sub> H <sub>13</sub> NO <sub>6</sub>	L-(+)-Threose, aldononitrile, triacetate
13	16.68	311.185	311.3	C <sub>18</sub> H <sub>17</sub> NO <sub>4</sub>	Fmoc-beta-alanine
14	16.86	207.139	209.67	C <sub>11</sub> H <sub>12</sub> ClNO	Vitamin K5 hydrochloride
15	17.15	257.175	259.3	C <sub>11</sub> H <sub>21</sub> N <sub>3</sub> O <sub>4</sub>	L-Glutaminyl-L-isoleucine
16	17.32	207.139	207.23	C <sub>11</sub> H <sub>13</sub> NO <sub>3</sub>	Glycine, N-(m-toluoy)-, methyl esterl
17	17.47	309.17	309.27	C <sub>11</sub> H <sub>19</sub> NO <sub>9</sub>	Sialic acid
18	18	271.191	272.4	C <sub>18</sub> H <sub>24</sub> O <sub>2</sub>	Estradiol
19	18.12	293.175	293.89	CaI <sub>2</sub>	Calcium iodide
20	18.96	277.18	278.114	I <sub>2</sub> Mg	Magnesium iodide

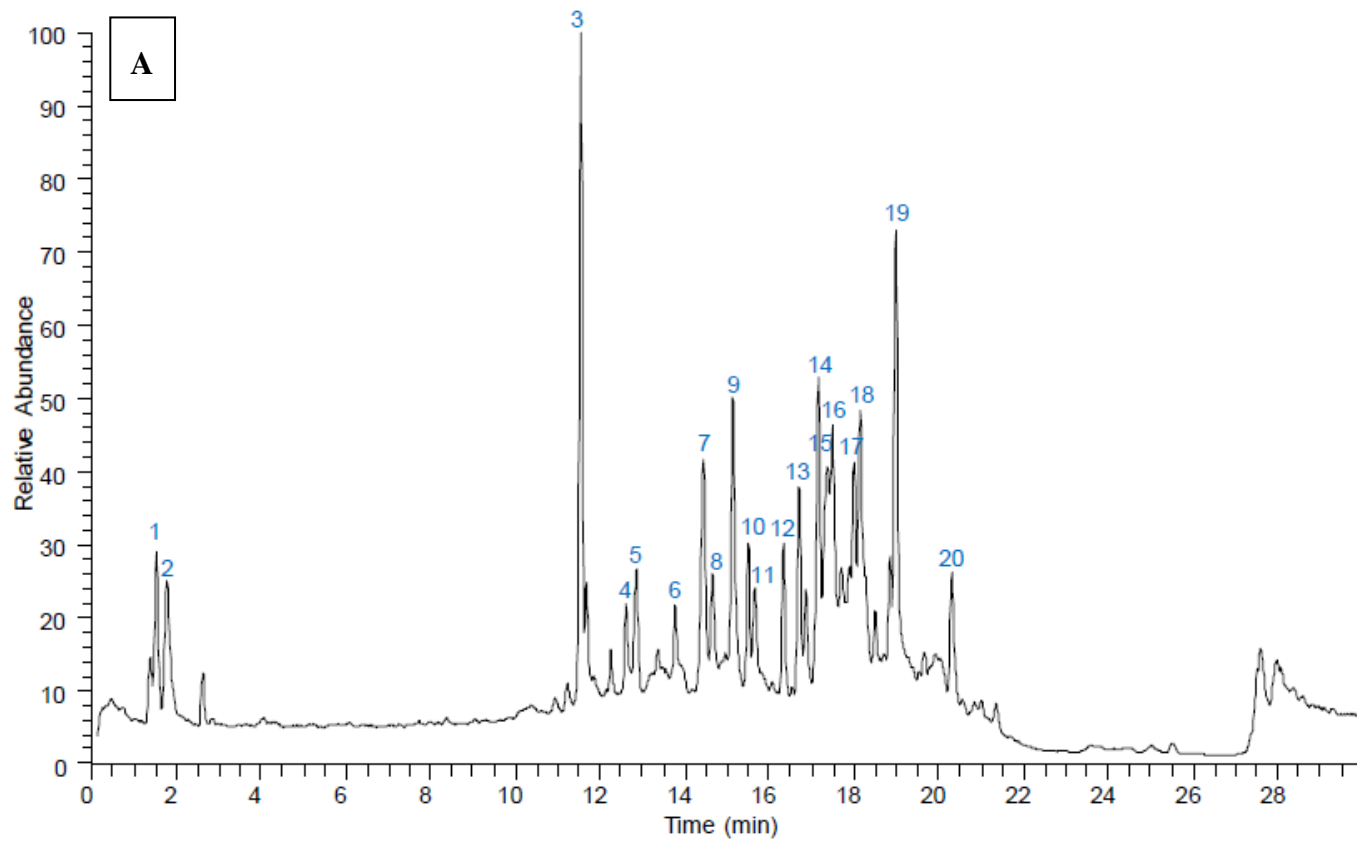
**Supplementary Table 3** Organ coefficient (wet weight of organ (g)/body weight (g)) x 100

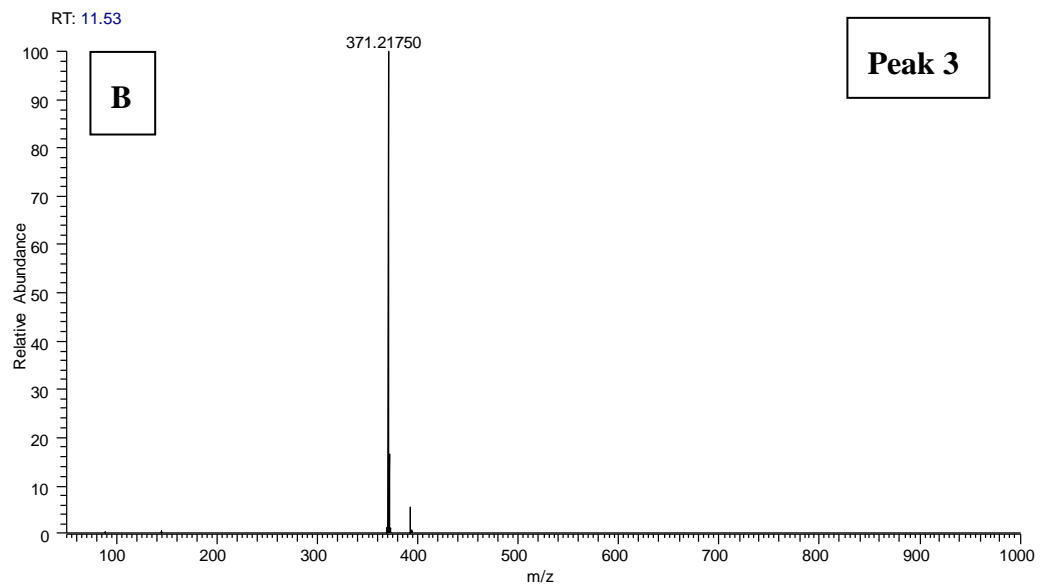
<b>Group/Organ Coefficient</b>	<b>Testes</b>	<b>Epididymis</b>	<b>Seminal Vesicle</b>
Control	0.39 ± 0.02	0.16 ± 0.01	0.29 ± 0.01
10 mg/kg BW/d EBN	0.27 ± 0.04	0.13 ± 0.01	0.30 ± 0.05
50 mg/kg BW/d EBN	0.31 ± 0.02	0.13 ± 0.01	0.35 ± 0.03
250 mg/kg BW/d EBN	0.32 ± 0.03	0.14 ± 0.01	0.32 ± 0.04

Data are presented as Mean ± SEM with  $n=4$ . No significant different was noted between the groups for all organ coefficient.

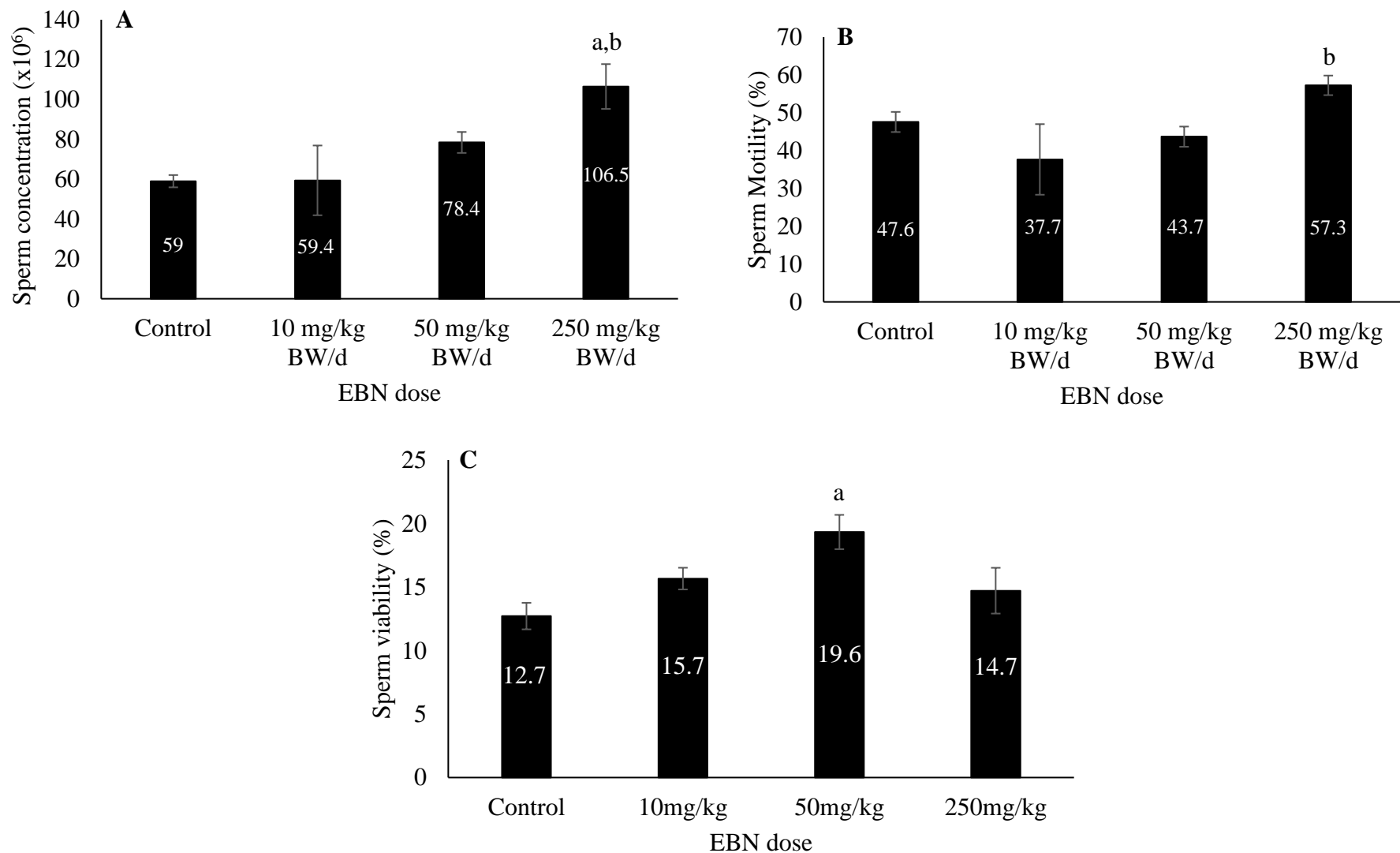
## Supplementary Figure

## Full Scan





**Supplementary Figure 1.** Chromatogram of EBN compound characterization through LC-MS  
A represents full scan chromatogram of Orbitrap LCMS analysis  
B represents the most intensive peak in the mass spectrum

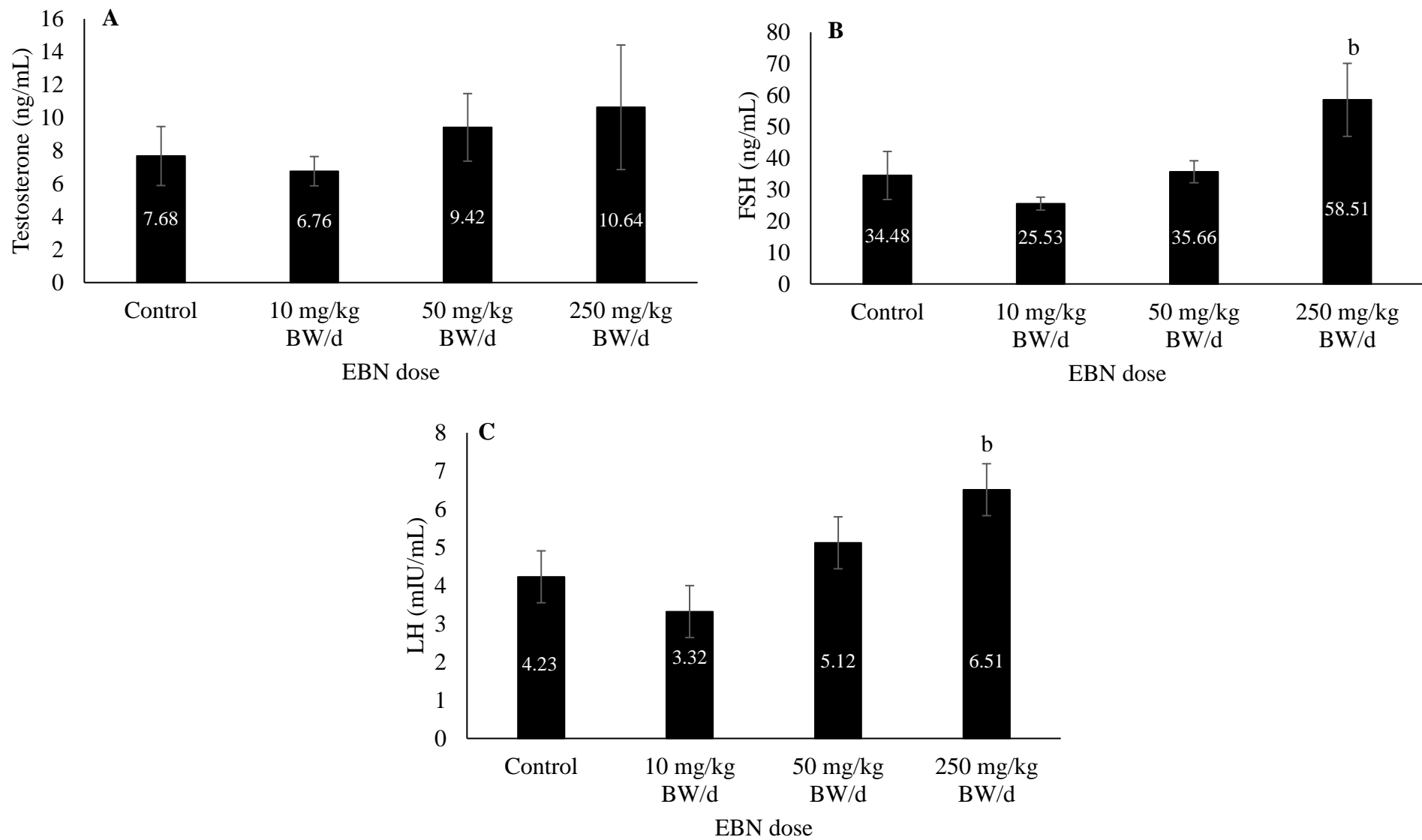


**Supplementary Figure 2.** The effect of EBN supplementation on sperm parameters.

A Sperm concentration with the respective dose of EBN treatment. Data are presented as Mean  $\pm$  SEM with n=4. <sup>a</sup> represents a significant difference compared to the Control group with  $p < 0.05$ ; <sup>b</sup> represents a significant difference compared to the 10 mg/kg BW/d EBN group with  $p < 0.05$ .

B Percentage of total motile sperm with the respective dose of EBN treatment. Data are presented as Mean  $\pm$  SEM with n=4. <sup>b</sup> represents a significant difference compared to the 10 mg/kg BW/d EBN group with p<0.05.

C Percentage of viable sperm with the respective dose of EBN treatment. Data are presented as Mean  $\pm$  SEM with n=4. <sup>a</sup> represents a significant difference compared to the Control group with p<0.05.



**Supplementary Figure 3.** The effect of EBN supplementation on serum hormonal level.



A Testosterone level in serum with a respective dose of EBN treatment. Data are presented as Mean  $\pm$  SEM with n=4. No significant difference between the groups was noted.

B FSH level in serum with a respective dose of EBN treatment. Data are presented as Mean  $\pm$  SEM with n=4. <sup>b</sup> represents a significant difference compared to the 10 mg/kg BW/d EBN group with p<0.05.

C LH level in serum with a respective dose of EBN treatment. Data are presented as Mean  $\pm$  SEM with n=4. <sup>b</sup> represents a significant difference compared to the 10 mg/kg BW/d EBN group with p<0.05.