# The Effects of American Ginseng Extracts on Streptozocin Induced Diabetic Sciatic Neuropathy in Exercised Sprague Dawley Rats

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# Agenda

- Background
- Hypothesis
- Experimental design
- Methods and Materials
- Preliminary results
- Conclusions

# Background

- Exercise and vascular well-being
  - Endogenous antioxidant defenses and heat shock protein expression
  - Blood flow
  - Shear stress
  - Neuro-activation

- Ginseng and diabetes
  - Expression of endogenous antioxidant enzymes (e.g. Cu/Zn superoxide dismutase)
  - Nitric oxide (NO) synthesis
  - Pro-inflammatory cytokines (e.g. TNF-a, IL-6, and C reactive protein[CRP])

DeBlieux,P.M.C., et al. (1993); Atalay, M, et al. (2003); De Angelis, K.L.D., et al (2000); Gillis, C.N.(1997)

# Background

- Diabetic Neuropathy (DN)
  - 346 million people➔50%
  - Etiology
    - Formation of advanced glycation end products
    - Increased oxygen free radical activity
    - Reduced endothelial nitric oxide activity
  - Pathogenesis of DN
    - Microvascular abnormalities
    - Endothelial dysfunction
    - Reduced nerve blood flow
    - Hypoxia ➔capillary damage ➔escalating hypoxia
  - Potential therapeutic approaches
    - To increase nerve blood flow Exercise&Ginseng
    - To prevent/reduce the generation of advanced glycation end-products Exercise&Ginseng
    - Treatment with antioxidant Exercise&Ginseng

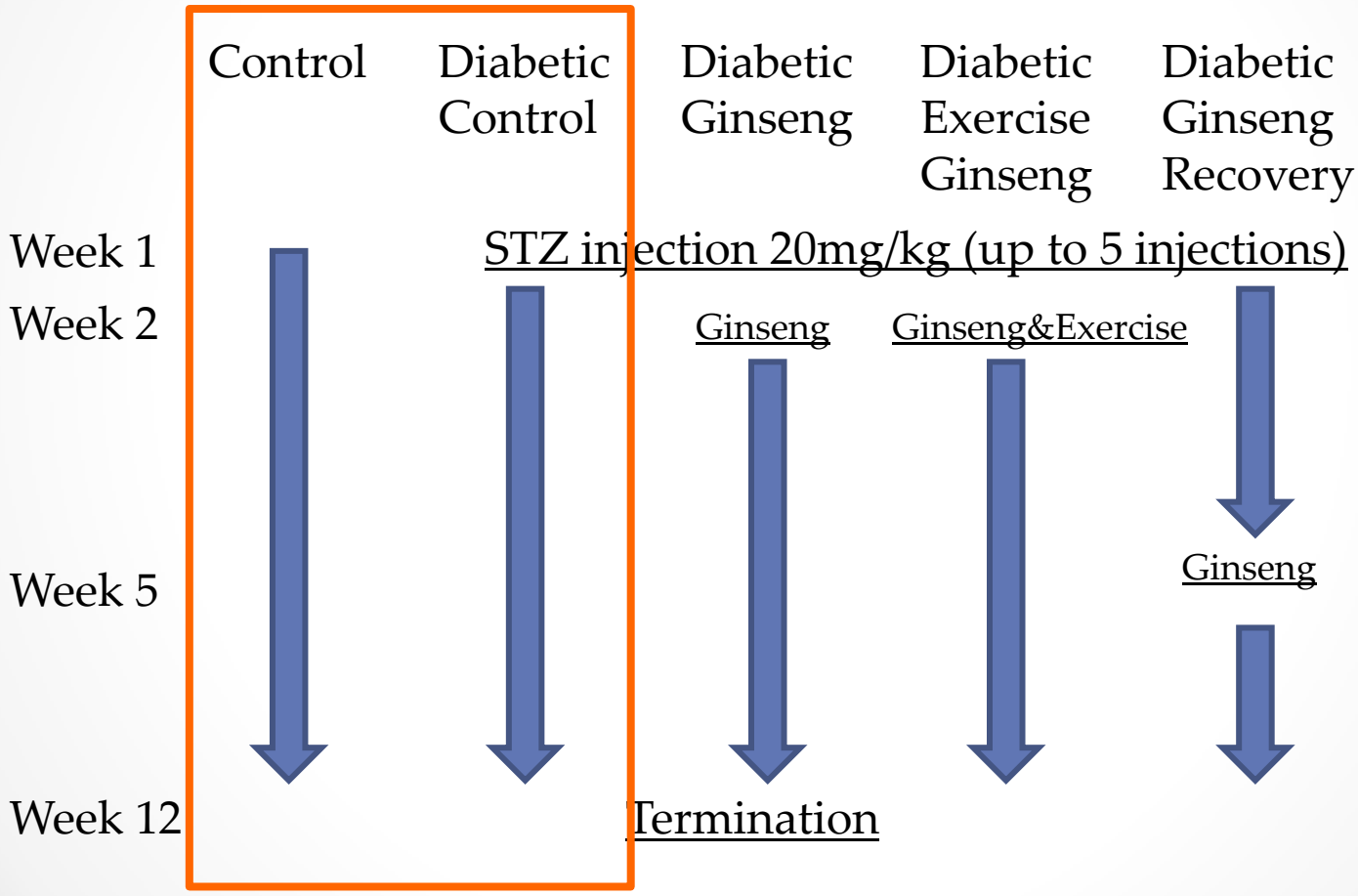
Low P.A. (1987); Tesfaye, S. et. Al (1994); Cameron N.E. & Cotter M.A. (2007)

World Health Organization

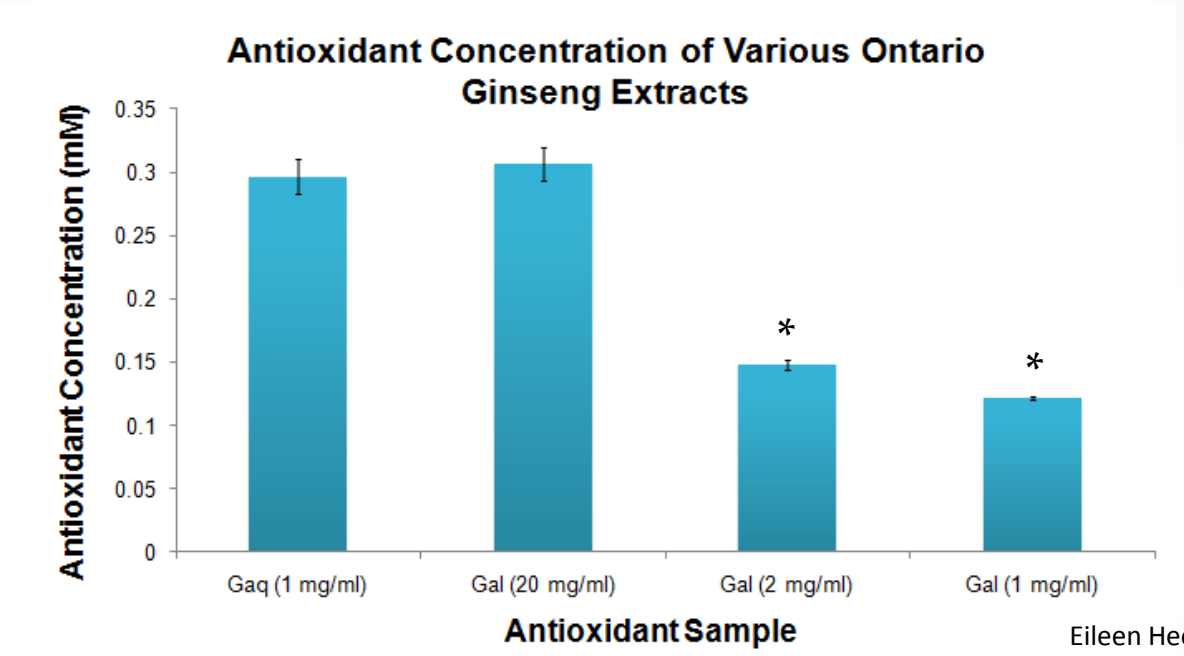
# Hypothesis

American ginseng aqueous extract (AQ-G) along with physical exercise attenuates/prevents diabetic sciatic neuropathy by maintaining endoneural vascular and nerve fiber integrity, and modulating nitric oxide pathway in STZ induced type-1 diabetic rats.

# Experimental design



# Experimental design




Eileen Heo (2011)

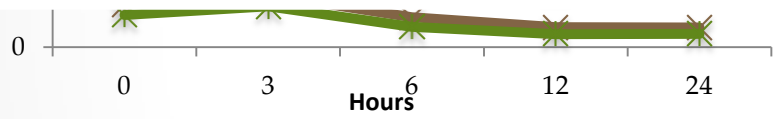
- Ginseng administration
  - American ginseng aqueous extract
  - Supplied with drinking water
  - 250mg/kg/day

# Methods and Materials

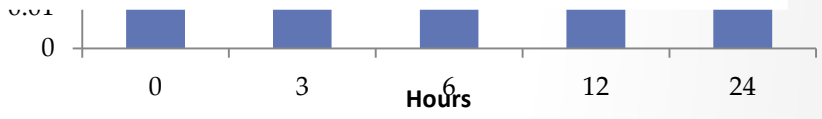
- SD male Rats (8-week old; 250-300g)
- Blood analysis
  - HbA1c
  - Lipid/lipoprotein analysis
  - Multiplex assay for pro-inflammatory cytokine such as CRP, TNF-a, and IL-6
- Total radical scavenging ability assay(TRSA)
  - Colorimetric reaction
  - Reported in mM Trolox equivalent
- Protein immunoblot/Immunohistochemistry
  - To examine endoneural vasculature, and endothelial dysfunction (molecular markers within NO related pathway)
- Histochemistry
  - H & E
  - Luxol fast blue (myelin specific)
  - Bielschowsky's silver stain (axon specific)

# Results

**Ginsenoside content breakdown over 24 hours**




**Radical scavenging ability of aqueous ginseng extracts**




- Re, Rg1, Rb1, Rc, Rd are the major ginsenoside species found in aqueous American ginseng extracts.
- The amount of detectable ginsenoside decreases overtime.
- The radical scavenging ability of aqueous American ginseng extracts is stabilized.

| Time \ Ginsenoside | Re | Rg1 | Rb1 | Rc | Rd | Total | Remaining |
|---|---|---|---|---|---|---|---|
| 0 Hr | 131.2792093 | 43.75973642 | 93.55609136 | 14.33546031 | 10.84260697 | 293.7731043 | 100% |
| 3 Hr | 123.3508181 | 41.11693936 | 87.19975688 | 17.68951572 | 13.76900341 | 283.1260335 | 96.37% |
| 6 Hr | 96.40065402 | 32.13355134 | 61.72468944 | 10.0900817 | 6.892943003 | 207.2419195 | 70.54% |
| 12 Hr | 61.92315067 | 20.64105022 | 39.90117392 | 6.803943585 | 4.445446316 | 133.7147647 | 45.51% |
| 24 Hr | 64.94763666 | 21.64921222 | 43.81845216 | 6.672308184 | 4.508636143 | 141.5962454 | 48.19% |

# Results

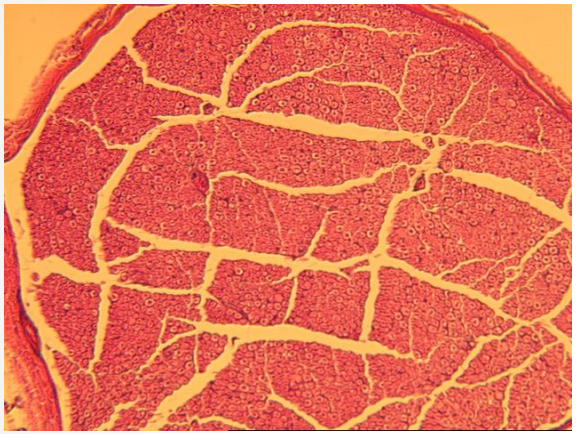
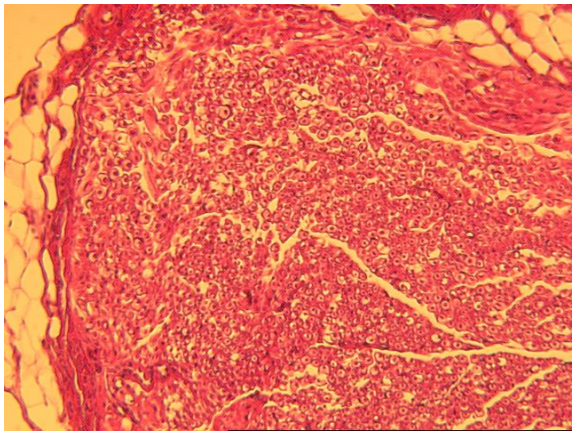
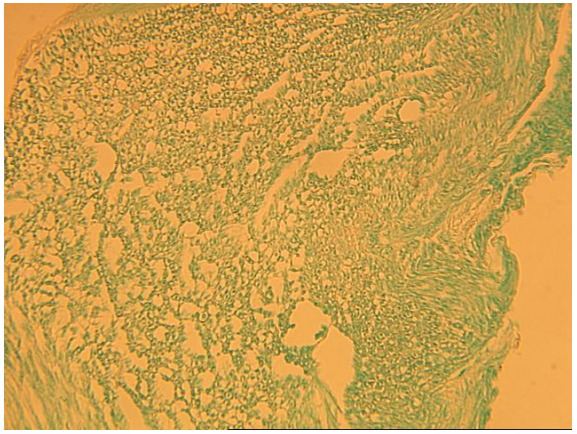
| | Glu. lvl(mmol/dL) @ week 6 | BW (g) @ week 6 | Glu. Lvl(mmol/dL) @ week12 | BW (g) @ week 12 |
|---|---|---|---|---|
| Control | 4.0 ± 0.5 | 496 ± 41 | 6.1 ± 1.0 | 619.6 ± 56.5 |
| Diabetic Control | 18.9 ± 3.7* | 350 ± 33* | 20.9 ± 3.4* | 376.9 ± 49.9* |

Mean with standard deviation are reported
Control (n=12); Diabetic control (n=16);
* Significant different $p<0.05$

| | HbA1C/Total Hb | Triglyceride (mmol/L) |
|---|---|---|
| Control | 5.39 ± 1.20% | 1.23 ± 0.43 |
| Diabetic Control | 8.18 ± 1.47%* | 10.25 ± 8.54* |

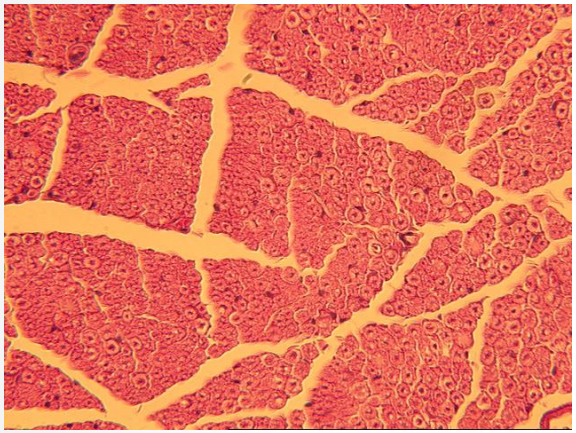
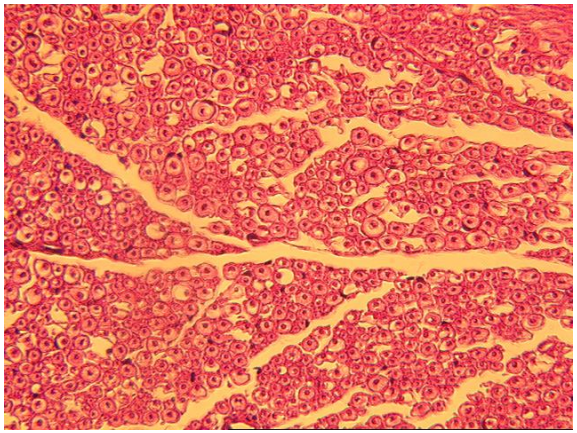
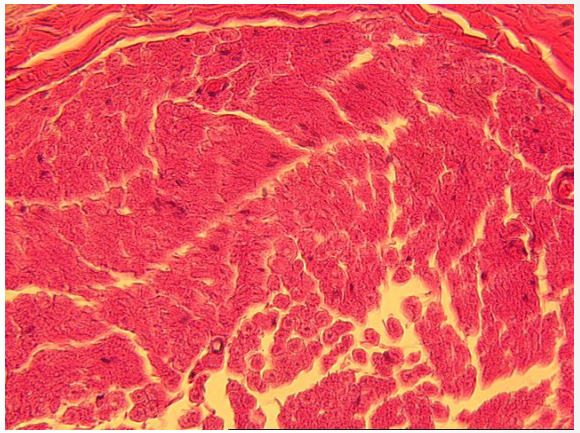
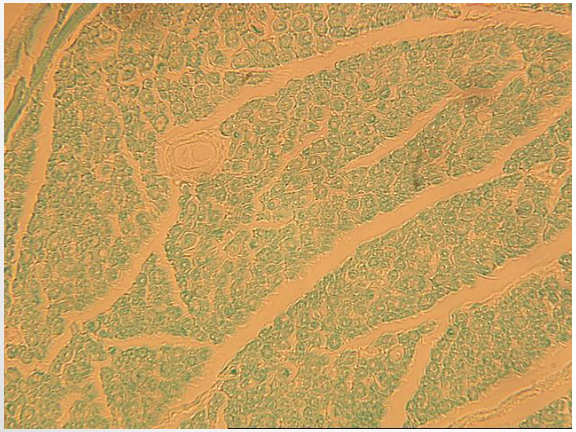
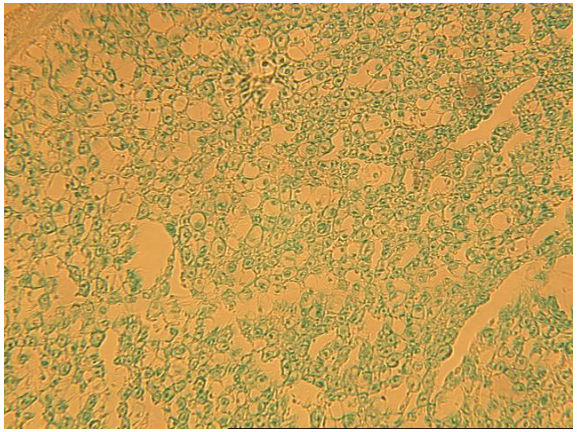
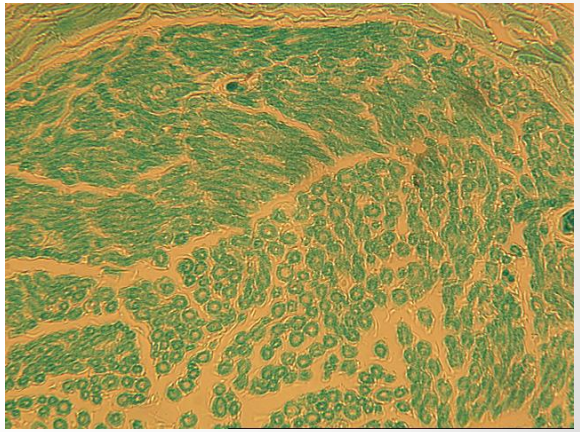
Mean with standard deviation are reported
Control (n=12); Diabetic control (n=16);
* Significant different $p<0.05$

# Results

| | Control | Diabetic Control | Diabetic + Ginseng |
|---|---|---|---|
| H & E |  |  |  |
| LFB |  |  |  |

- 200x

# Results

| | Control | Diabetic Control | Diabetic + Ginseng |
|---|---|---|---|
| H & E |  |  |  |
| LFB |  |  |  |

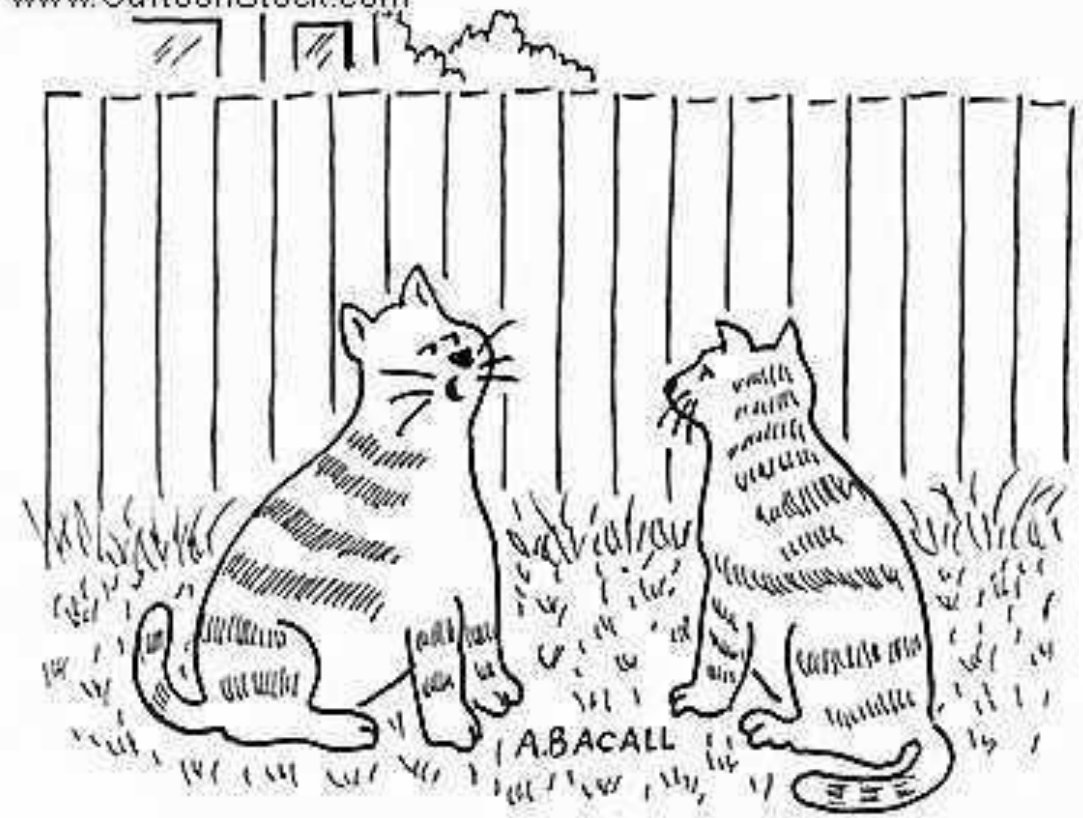
- 400x

# Conclusion

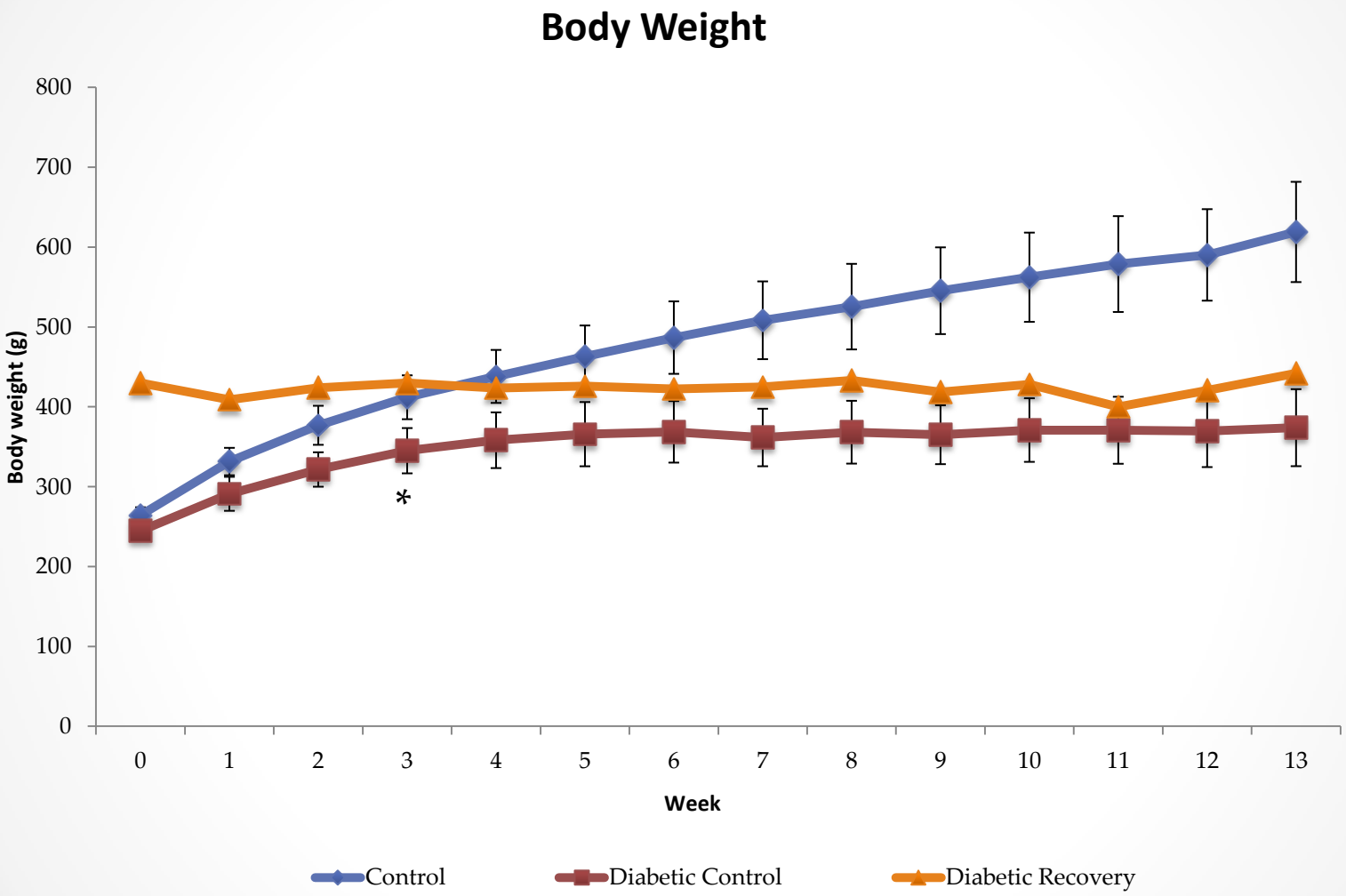
- So far…..
  - The amount of detectable ginsenosides  decreases overtime.
  - However, the potency of radical scavenging ability of aqueous ginseng extracts is unaffected by time.
  - To confirm diabetes,
    - Plasma glucose level, body weight,  endpoint glycated hemoglobin level,  triglyceride level are all significantly different between Control and Diabetic Control group.
  - From the neural morphology point of view,
    - Reduction of the myelination in diabetic control group
    - Sign of endoneural edema

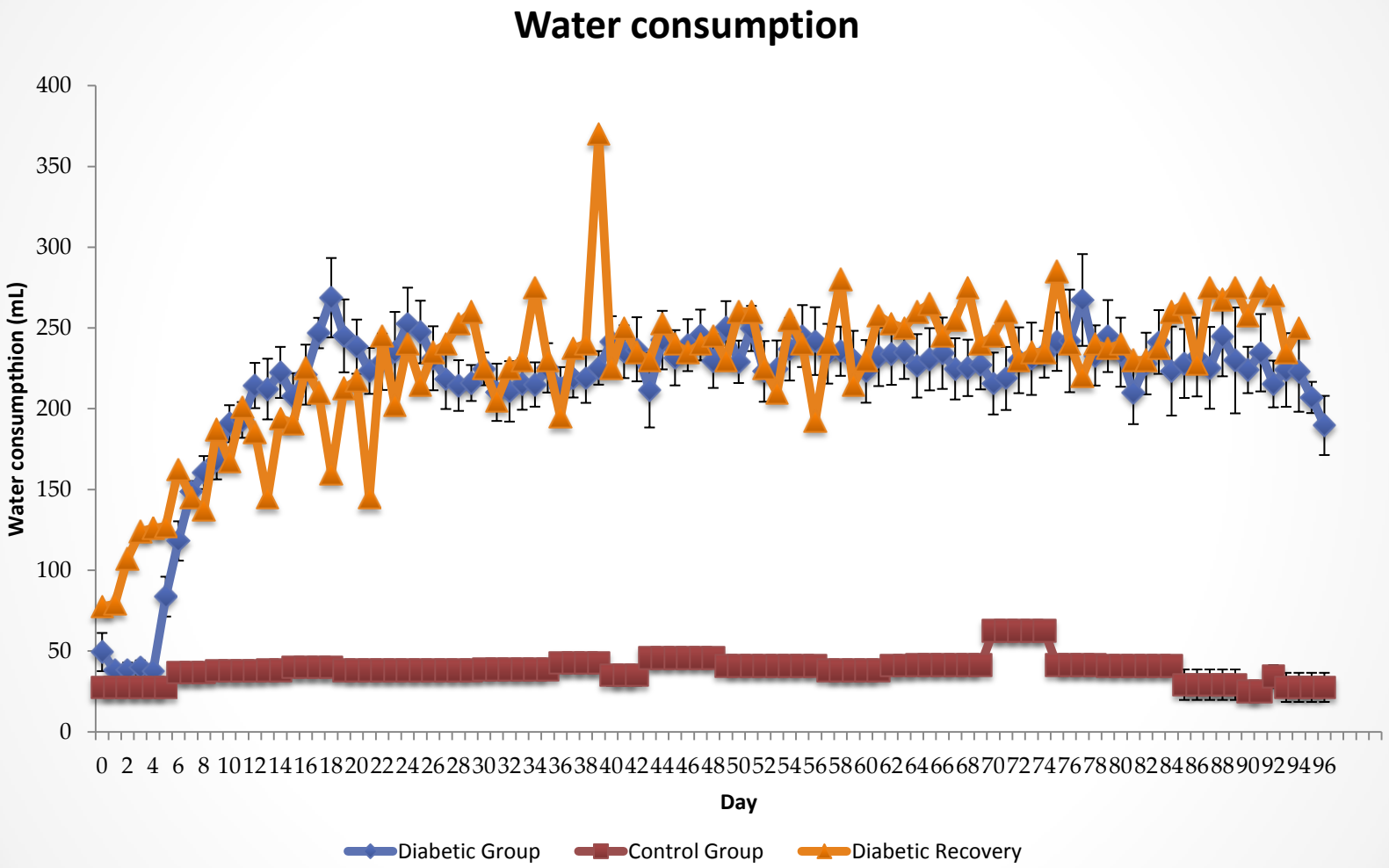
# Conclusion

- We believe that the model we adopted is adequate to study diabetic peripheral neuropathy, and it has set up a reliable reference point for the future study.




"I take lots of antioxidants. That's why I'm still on the first of my nine lives."


Body Weight
Body weight (g)
800
700
600
500
400
300
200
100
0
0
1
2
3
4
5
6
7
8
9
10
11
12
13
Week
*
Control
Diabetic Control
Diabetic Recovery


Water consumption
Water consumpthion (mL)
400
350
300
250
200
150
100
50
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50 52 54 56 58 60 62 64 66 68 70 72 74 76 78 80 82 84 86 88 90 92 94 96
Day
Diabetic Group
Control Group
Diabetic Recovery