**Aspalathin, a natural product with the potential to reverse hepatic insulin resistance by improving energy metabolism and mitochondrial respiration**

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**Material and methods**

**Western blot analysis**

For Western blot analysis, membranes were probed overnight at 4°C with the relevant primary antibodies glucose transporter (GLUT)2, protein kinase B (AKT, p-AKT (Ser 473), 5' AMP-activated protein kinase (AMPK, p-AMPK (Thr172), carnitine palmitoyltransferase 1 (CPT1) and phosphoinositide 3-kinase (PI3K, p-PI3K (p85)) , as well as horseradish peroxidase (HRP) conjugated secondary antibody (β-actin) applied for 1.5h the following day. Chemiluminenscence using a Chemidoc-XRS imager and Quantity One 1-D software (Biorad Laboratories, Hercules, CA, USA) were used detect and quantify proteins. β-actin was used as the reference control.Three independet experimnents were conducted and one blot was selected as a representative. The selected represenative image was croped and image was used to quantify molecular weight size. In some of the the pictures out of 8 lanes loaded only 6 were used as the other treatment [gree rooibos extract (GRE) and green rooibos extract + insulin (GRE+I)] is not discussed in the current article. Molecular weight and band analysis was quantified using image J software.

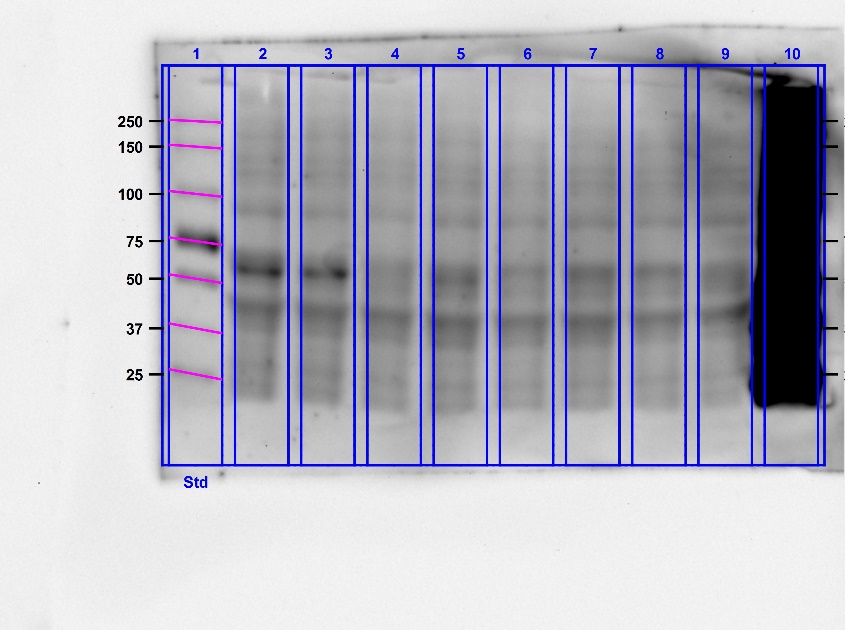
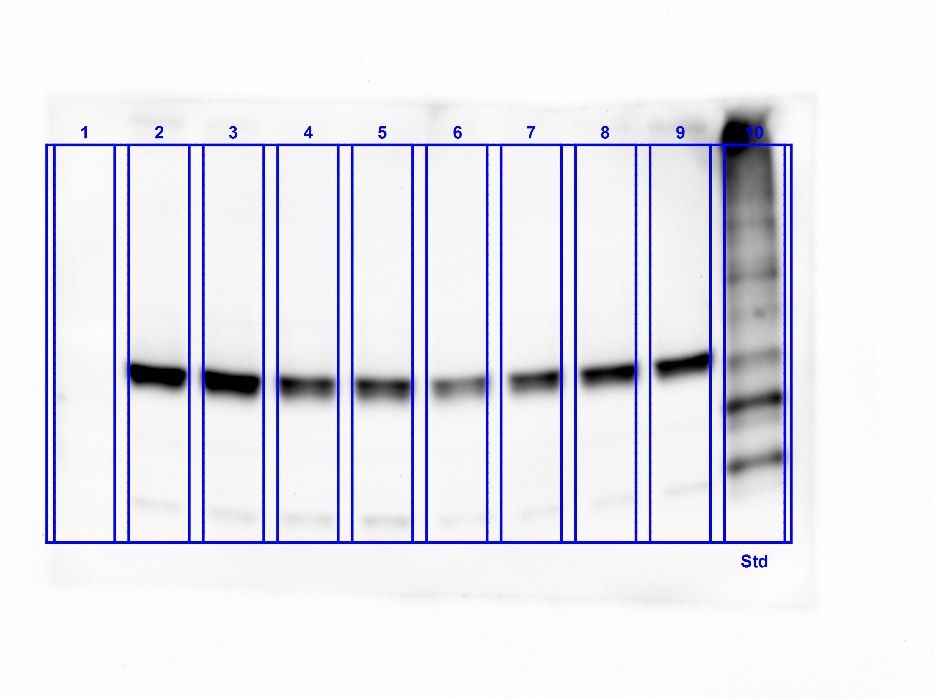
**Protein kinase B (AKT) original Western blots**

Three independent experiments were conducted for both phosphorylated (pAKT) and total AKT (tAKT), and in all figures, GRE and GRE+I were run on the same blot. However, GRE and GRE+I is not discussed in this paper. Similar tread was attained in all blots (Fig.2A-F). Experiment 2 (Fig.2C and D) was cropped and used as a representative as shown in (Fig. 2G).

A B

Marker kDa G GI P PI GRE GRE+I ASP ASP+I Marker kDa

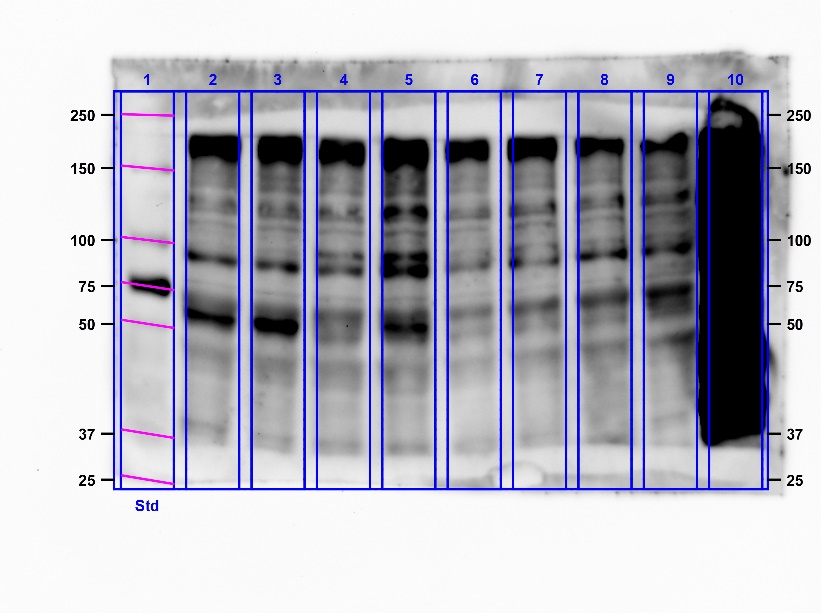
Marker kDa G GI P PI GRE GRE+I ASP ASP+I Marker kDa



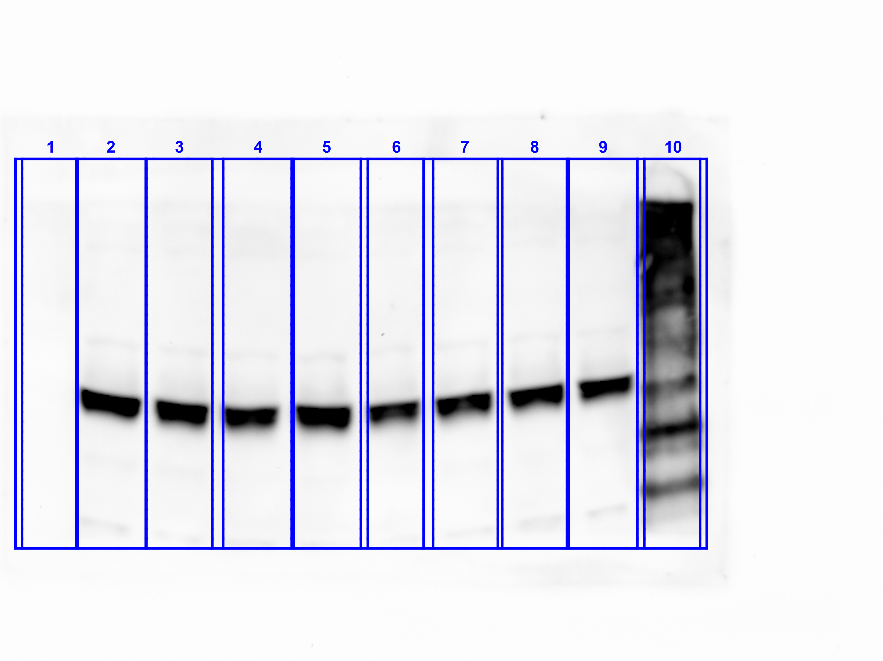
~60 tAKT

~60 pAKT

C D



~60 pAKT



~60 tAKT

Marker kDa G GI P PI GRE GRE+I ASP ASP+I Marker kDa

Marker kDa G GI P PI GRE GRE+I ASP ASP+I Marker kDa

E F

Marker kDa G GI P PI GRE GRE+I ASP ASP+I Marker kDa

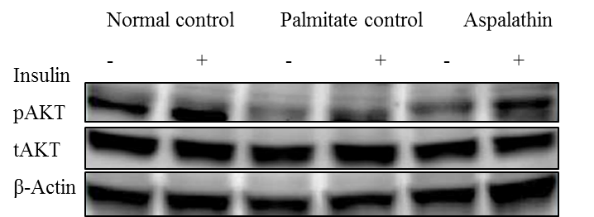
C:\Users\smazibuk\AppData\Local\Microsoft\Windows\INetCache\Content.Word\takt exp 2 original blot.tifC:\Users\smazibuk\AppData\Local\Microsoft\Windows\INetCache\Content.Word\Akt Exp 1 original blot.jpg.tif

Marker kDa G GI P PI GRE GRE+I ASP ASP+I Marker kDa

~60 tAKT

~60 pAKT

G



G GI P PI ASP ASP+I

**S2 Data set.**