Protective Effect of Beta-Sitosterol against Ethanol Toxemia

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Abstract:

Herbal medications have no indications of interactions with alcohol on their labels. There has been at least one clinical finding that saw palmetto with active ingredient beta-sitosterol used for enlarged male prostates caused pancreatic damage. This study with 20 male mice hypothesized that ethanol would increase the toxicity of beta-sitosterol. It was found that mice dosed by gavage with 0.1 ml of 95 proof ethanol died starting at the second dose and increased to 100% by the sixth dose (unexpected based on literature). 60-microgram injections of beta-sitosterol prevented deaths in 80% of the mice past the sixth dose opposite to what was expected. Beta-sitosterol failed to protect the liver, enlarged the pancreas, and protected the kidneys from ethanol induced damage.

Introduction:

Large quantities of herbal medications of unknown content claim medical properties, these are available over-the-counter or on the Internet. Saw Palmetto (*Serenoa repens*), a plant, is sold to older men with enlarged prostates (benign prostatic hyperplasia or BPH) or anticipating BPH that contains this herb or one of its purified active ingredient. One of the ingredients of Saw Palmetto is beta-sitosterol which has been shown to be pharmacologically active (1). However, they are not warned about possible toxicity/side effects.

A published clinical study of a 65 year old male with a variety of health problems and heavy alcohol use developed pancreatitis (inflammation and dangerous deterioration of pancreatic cells) one week after treatment with Saw Palmetto (2). The doctors did not believe alcohol was the leading cause of pancreas damage as indicated by very high blood (and urine) sugar levels which indicated the insulin producing cells of the pancreas were damaged. This is confusing because alcohol intake also causes pancreatic and liver damage (3). The hypothesis of the study is that mice given ethanol and beta-sitosterol will have decreased liver and pancreas function as determined by increased urine glucose (pancreatic damage) or urine bilirubin (causes jaundice color that indicates liver damage [4]).

Materials and Methods:

Twenty male mice of the strain C57/Bl6 ages 6-13 weeks were weighed and separated into four age-balanced groups of five mice. The groups of mice were then placed into cages that had water and food freely available. The animal protocols were approved by the Minnesota State University Institutional Animal Care and Use Committee (IACUC) approval number 16-06

Mice dosed twice per week on Mondays and Wednesdays with 0.1 ml 0.849% NaCl (oral saline by curved gavage needle), 0.1 ml sterile corn oil i.p. (abdominal injection) as the control; 0.1 ml Saline, 0.1 ml 60 µg Beta-Sitosterol in corn oil, 0.1 ml 0.425% NaCl plus 95 proof ethanol (oral ethanol), i.p. corn oil; and 0.1 ml oral ethanol, 0.1 ml i.p. beta-sitosterol. These groups were selected to give clarity on how the compounds, ethanol and beta-sitosterol, interacted individually and in combination. Mice euthanized after week eight when one control mouse died. Oral dosing was based on a previous study that was investigating deleterious effects of ethanol (5). Abdominal dosing by injection was based on a similar study in mice (6).

Urine collected on Fridays and analyzed with VWR urine test strips. Initially the urine was analyzed for glucose (pancreas damage), ketones (near death or ketoacidosis from pancreas damage), bilirubin, urobilinogen (liver damage and kidney damage) and protein (kidney damage prior to death). After mice started to die from the treatments, the urine analysis was expanded to test for pH (acidosis), and leukocytes (white blood cells in urine) in addition to the original five compounds. Urine was collected from the mice by spinning the by the tail and then picking the mice up by the tail and scruff. The mice were then placed over a funnel and gentle pressure was applied to the lower abdomen.

Mice dissected immediately after euthanization or frozen after death, weighed and dissected following thawing and organ data collected. Organ analysis was performed on the liver, intestines, pancreas, kidney and heart. Organs were weighed and inspected for condition.

Two-way ANOVA was run using SYSTAT 9.0® software with $P<0.05$ accepted as statistically different.

Results:

There was no statistical difference in the body weights of the treatment groups as determined by two-way ANOVA and seen in Table 1.

Table 1. Mean±SEM body weights of treatment groups.

Treatment Groups	Body Weights
Saline and corn oil	25.456±0.890
Saline and beta-sitosterol	26.100±1.069
Ethanol and corn oil	23.273±2.398
Ethanol and beta-sitosterol	25.042±0.506

Mortality was seen in the two treatment groups that were dosed with ethanol following the second dose. In the ethanol treatment group, ethanol and corn oil, two mice died after dosing and one mouse died in the ethanol and beta-sitosterol group as seen in Figure 1. Mortality increased after each subsequent dose in the ethanol treatment group until all mice were dead, after dose six. One mouse in the control group died in week eight whereupon the study was terminated.
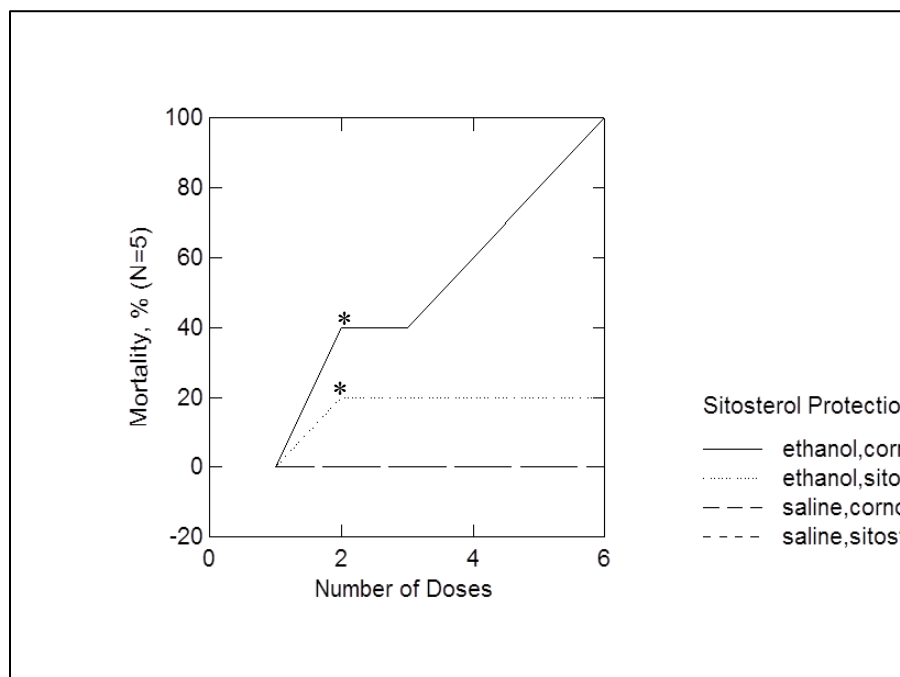

Figure 1. Mortality of treatment groups based on dose number. * denotes different from control p<.05 (LSD posthoc following significant effect of ethanol and beta-sitosterol effects). Ethanol mortality unexpected based on published study results (3).

Following dissection the intestines were weighed and visually inspected for appearance and condition. Statistical analysis of the intestinal weights indicated a statistical difference between groups treated with ethanol and groups not treated with ethanol as seen in Figure 2.

Visual inspection of the intestines revealed distension of the intestines in groups treated with ethanol.

The livers of the mice in the ethanol treatment groups were visually dark red in color with dark spots throughout the tissue. There was a statistical difference between groups treated with ethanol and groups not treated with ethanol as determined by a two-way ANOVA and as seen in Figure 2.
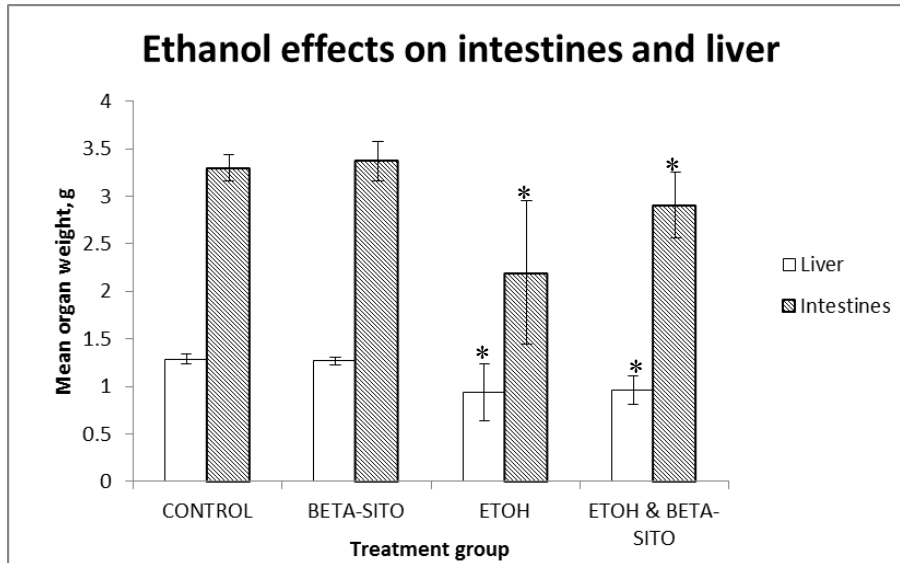

Figure 2. Ethanol showed expected negative effects on liver (damage) and intestinal weights (distended but lacking brush border) compared to control mice. * denotes $p<.05$ compared to control (ethanol effect alone). Error bars = SEM.

Visually there was no noticeable difference in pancreases other than size, which is accounted for based on the weight data obtained. Statistical analysis showed a statistical difference in the weights of pancreases in groups treated with beta-sitosterol compared to groups not given beta-sitosterol, as seen in Figure 3.
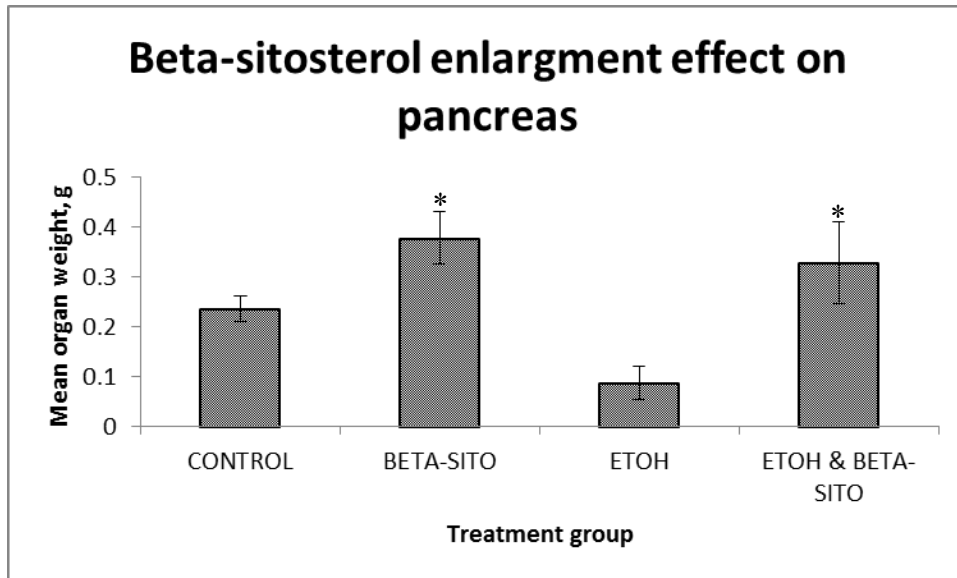
Figure 3. Beta-sitosterol increased pancreas size. * denotes p<.05 (beta-sitosterol effects alone).Error bars = SEM.

Ethanol reduced kidney weight. This result was not as pronounced in the beta-sitosterol and ethanol treatment group. Statistical analysis showed an interaction between ethanol and beta-sitosterol, as seen in Figure 4.
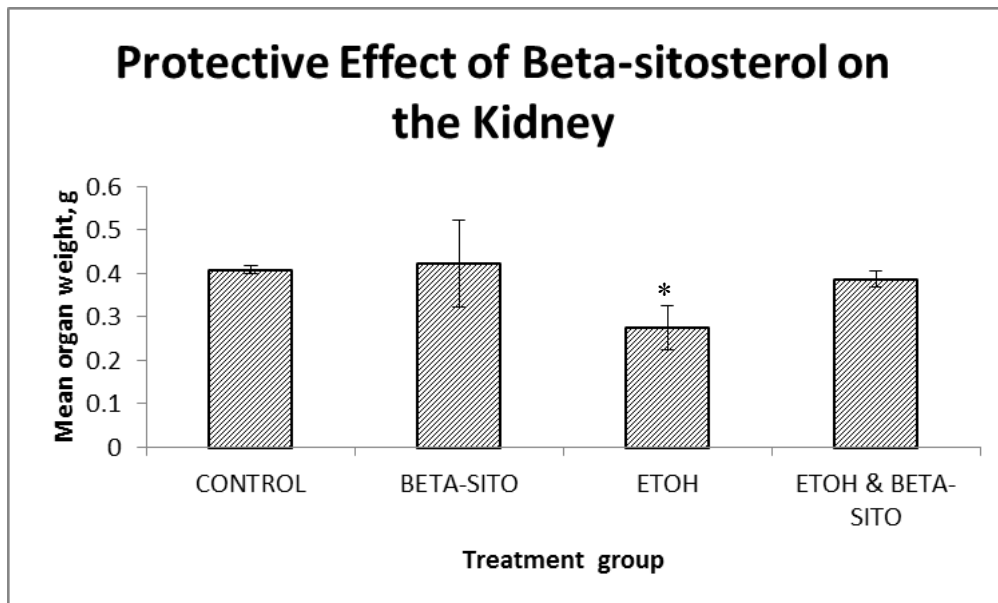

Figure 4. Interactions of Ethanol and Beta-sitosterol on the kidneys. * denotes p<.05 different from control and ethanol and beta-sitosterol dosed mice (ethanol*beta-sitosterol interaction term meaning beta-sitosterol prevented kidney damage). Error bars=SEM

Discussion:

Ethanol binge-like drinking caused intestine and liver damage and kills mice as expected. The mechanism of intestinal distension was not investigated by this study. The mice which were treated with ethanol showed signs of liver damage, which is consistent with current literature.

Beta-sitosterol caused an enlargement of the pancreas. This is expected if it represents hypertrophy due to damaging inflammation. This contradicts previous studies which indicated beta-sitosterol as an anti-inflammatory compound (7). However, the vast majority of studies involving beta-sitosterol have been focused on the prostrate and it is currently unknown if the anti-inflammatory property extends to other organs as well. As glucose administration is the only method to prevent alcohol toxemia in humans due to hypoglycemia, pancreatitis may explain why mice were protected from ethanol. This result needs future experiments to check blood glucose and insulin levels as the urinalysis was inconclusive except for higher protein and ketones in dying mice.

Beta-sitosterol mitigated the kidney damage by the ethanol. This was determined through statistical analysis. This is unexpected but may be a key factor in mice surviving ethanol. Phytohormones, such as beta-sitosterol, have been shown to prevent renal dysfunction (7,8). This was not the focus of this study. Future studies are needed to investigate the mechanism of kidney protection.

Decreased mortality was seen in the beta-sitosterol and ethanol treatment compared to the ethanol treatment. This result was interesting as increased mortality was expected. This result shows that data reveals unknown mechanisms.

Acknowledgements:

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