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*Chapter 5*

# BLUEBERRY EXTRACTS PROTECT AGAINST GROSS MOUSE FETAL DEFECTS INDUCED BY ALCOHOL TOXICITY

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## ABSTRACT

Alcohol is a powerful teratogen, systematically affecting prenatal development as well as postnatal functioning in humans and other mammals. Using a mouse model, this study explored the potential effects of anthocyanins from blueberry extracts in protecting against alcohol-induced prenatal developmental deficiencies.

Swiss mice were assigned to three experimental groups: control (CO), binge alcohol (BA) and alcohol-anthocyanin (AA). CO mice were administered normal saline (0.03 ml/g maternal body weight), while BA and AA mice received alcohol (25% v/v of absolute ethanol in normal saline at 0.03 ml/g maternal body weight), through intraperitoneal injections on days 5 and 7 following impregnation. Supplemental anthocyanins via blueberry extracts (0.03 mg/g maternal body weight)

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were additionally administered to the AA group, through subcutaneous-neck injections on days 0, 5, 7 and 12. Maternal mice were necropsied and fetuses removed at day 15 of gestation.

Statistical analysis ($p < 0.05$) showed that 15 day old mouse fetuses with prior exposure to binge alcohol with anthocyanin supplementation (AA) were partially protected from some gross developmental deficiencies over the binge alcohol fetuses (BA). Group comparisons (CO vs BA vs AA) showed significant fetal gross body differences in regards to average body weight (197 vs 90 vs 162 mg, respectively), crown-rump length (11.2 vs 9.1 vs 10.7 mm, respectively), liver surface areas (6.9 vs 2.5 vs 5.1 $mm^2$ respectively) and telencephalon (forebrain) surface areas (3.18 vs 1.47 vs 2.75 $mm^2$ respectively).

Results support the hypothesis that properties found in blueberry extracts serve to mitigate certain gross anatomical effects in mouse fetuses due to maternal binge alcohol exposure during prenatal development.

**Keywords:** anthocyanins, binge alcohol, fetus, mouse, telencephalon, liver

# INTRODUCTION

## Alcohol and Development

Pregnant mothers, who consume excessive alcohol, danger their developing embryos/fetuses. As Goodlett and Horn report, alcohol exposure during development increases oxidative stress, interferes with the activity of growth factors, and changes the regulation of gene activity [1]. The consequences of sustained maternal alcohol consumption can lead to abnormalities in the mental and physical development of the offspring. The abnormalities emerge from alcohol's effects on migration and differentiation of germ layers during the early embryonic period [2]. The sensitivity of early developmental stages insures that teratogen introduction will affect multiple natural developmental processes [3]. Abnormalities resulting from disturbed cellular migrations and differentiation include, but are not limited to, stunted growth, facial anomalies, and neurological damage in the central nervous system (CNS).

## Alcohol Effects on Prenatal Mice

Alcohol administration in prenatal mice alters intracellular signaling necessary for proper differentiation, leading to malformations, neurological defects and, ultimately, cell death [4]. Additionally, alcohol lowers the overall embryo size, suggesting that perigestational alcohol exposure induces abnormal blastocyst growth, impairment of embryo-trophoblastic growth, and expansion during implantation [5].

## Alcohol Effects on Developing Nervous System

Alcohol is particularly dangerous to the developing nervous system, since it has been shown to induce apoptosis in the cranial neural crest of embryos [6]. Research on craniofacial malformation confirms that ethanol exposure during gastrulation deforms the structure of the neural plate, a vital structure needed in the development of the neural tube and the later spinal cord [7]. Alcohol exposure affects brain development through numerous pathways at all stages from neurogenesis to myelination. Problems that occur during neurogenesis ultimately lead to both behavioral and motor control abnormalities [8]. Studies of cell migration and ethanol exposure show that ethanol exposure to organisms will initially increase the number of cells in the marginal zone and cortical plate, which will form the surface layers of the neocortex. However, this proliferation of cells becomes prone to early cell death, which will later lead to smaller numbers of cortical cells present in the brain as well as to a thinner cortex at later stages of development [9]. Supportive studies [10] on ethanol exposure to developing cerebral cortex demonstrate that brain volume, isocortical volume, isocortical thickness, and isocortical surface area of rats exposed to ethanol were significantly smaller than those regions in rats without ethanol exposure. Cerebral cortical cell numbers and morphology in primary sensory areas exhibit high sensitivity to ethanol exposure [11]. Embryonic brains of ethanol exposed mouse embryos at day 10.5 of development (about the mid-point of the mouse gestation period) show clear holoprosencephalic dysmorphic changes [12]. These abnormal changes in the prosencephalon area of the brain include small and incompletely divided telencephalic vesicles as well as anteriorly shifted and connected nasal pits. Additionally again in the embryonic mouse model, ethanol is responsible for irregular migration of pluripotent cells and, thus can cause a variety of deformities, especially to the developing spinal cord [13].

In summary, research strongly indicates that ethanol exposure hinders central nervous system (CNS) formation. Overall, damage to the CNS can result in learning difficultly, attention deficits and poor cause and effect reasoning. Damage to the development of the frontal lobes, which form from the telencephalon area of the brain, can be particularly harmful, since the offspring will be more likely to engage in dangerous behaviors as a result of prenatal alcohol exposure.

## Alcohol Effects on Liver Development and Function

Adult human binge drinking is a concerning trend that is exhibited more in liver pathologies than chronic alcohol consumption [14]. In the murine model, binge drinking results in the down- regulation of Hdac 1,7,9,10 and 11, while up-regulating Hdac 3, leading to alcohol-induced microvesicular hepatic steatosis and damage due to increased hepatic triglycerides [15]. In a subsequent study, the up regulation of Hdac3 down regulates cpt1α contributing to hepatic steatosis [16]. Additional research shows the importance of tumor necrosis factor (TNF)-α in alcohol-induced liver injury through the TNF-R1 pathway. Adult TNF-R1 knockout mice demonstrate no hepatic pathology, detailing the importance of TNF-α in the onset of steatosis and inflammation [17]. The transcription factor, early growth response (Egr)-1, has also been shown to contribute to steatosis development following acute ethanol exposure in mice [18]. Damage to the development of the liver may affect gene expression and lipid metabolism in adult male mice which had been injected with acute doses of ethanol [19].

Chronic alcohol exposure leads to hepatotoxicity through redox manipulations, which alter reactive oxygen species and reactive nitrogen species or glutathione concentrations inducing apoptosis and necrosis [20]. Chronic alcohol exposure in adult mice increases triacylglycerols in the liver leading to a fatty liver, while decreasing their concentrations in epididymal and subcutaneous white adipose tissues [21]. Liver steatosis also arises partially from factors such as increased formation of $NADH^{+}$ which allows for reduced coenzyme for fatty acid synthesis [22]. Together these factors provide detail as to the danger of both acute and chronic alcohol exposure on the liver.

## Health Benefits of Blueberries

Many consumers are aware of the health benefits that fresh blueberries provide [23]. These include basic nutrient properties, antioxidant activity, [24, 25] anti-aging properties, [26] cancer prevention, [27, 28] protection against age-related neurological defects, [29] urinary tract health, protection against diabetes, [30] and cardiovascular health [31]. The polyphenolics and anthocyanins, found in ripened blueberries, are the primary health promoters and protective antioxidant agents [32] In comparison to many other fruits, blueberries contain higher levels of protective anthocyanins. These benefits are based on various studies, many of them animal studies where the findings have been superimposed on humans. This list has also been a clarion marketing call and elicited many consumers to choose blueberries for consumption rather than other fruits which have lower levels of antioxidants.

What if the health benefit list of blueberries or blueberry anthocyanins could be expanded? This chapter, based on preliminary research in our laboratory, documents that blueberry anthocyanins in the form of blueberry extract can alleviate some of the teratogenic influences of maternally ingested alcohol on embryonic/fetal development. That claim, if further verified, has huge implications. The idea that anthocyanins protect against some of alcohol teratogenic influences has been noted by several other investigators in other biological systems; [33, 34] however to date, except for this model project, that connection has not been demonstrated in an *in vivo* mammalian developmental system.

## Anthocyanin Interaction with Alcohol

Anthocyanins are natural pigments present in blueberries that belong to the flavonoid class of compounds. A primary antioxidant compound found in blueberries is cyanidin-3-glucoside (C3G). Research reveals that C3G is capable of reducing neurodegenerative effects of ethanol exposure by alleviating oxidative stress [35]. When bowel disease is induced in mice with trinitrobenzene sulfonic acid (TNBS), the experimental groups that received dietary blueberries along with TNBS had lower risk of induced bowel disease than that of control groups [36]. Anthocyanin administration partially eradicates free radicals from superoxide, peroxide, hydrogen peroxide, and hydroxyl groups, which are responsible for the toxic responses to ethanol in fetal tissues [37]. Anthocyanins also reduce DNA damage, which is a major

indicator in Fetal Alcohol Syndrome [38]. Similarly the antioxidant, vitamin E, alleviates oxidative stress in ethanol exposed neonatal rats [39]. Since identifying protective properties is important for creating therapeutic treatments, these studies promote the role of antioxidants as preventive measures against alcohol-induced damage.

Antioxidants have the power to shield against free radicals in the body that can harm fetal cells. Lack of research, however, has slowed the transition of antioxidants from research labs to the clinical field, as a medicinal treatment. Mouse developmental studies can provide this transitional bridge. Mouse gestational day 9 is comparable to human gestational day 20 in which the neural plate begins to fold over the notochord. Mouse gestational day 11 is comparable to human gestational day 30 in which the forebrain, somites and 1st, 2nd and 3rd pharyngeal arches are present. Mouse gestational day 15 is comparable to human gestational day 55 in which the limbs, trunk, heart, liver and even features of the face can be identified [40].

## MODEL PROJECT

### Experimental Objectives

The teratogenic effects that binge alcohol alone can have on a developing embryo or fetus are well documented in various model systems. However, the potential protection of anthocyanins against this alcohol toxicity has not been examined in developmental model systems. Consequently our project approach used an *in vivo* mouse development model to inspect the extent of the protection that anthocyanins provide in combating the life-threatening effects of oxidative stress on embryos and fetuses from alcohol induction exposure during gestation. To investigate the extent of gross anatomical malformations, three experimental groups of pregnant mice were used: control (CO), binge alcohol (BA), and binge alcohol supplemented with anthocyanins (AA). The goal for this experiment was to clearly demonstrate the protective role of anthocyanins against the teratogenic influences of alcohol as shown in gross anatomic parameters – whole body, forebrain, and liver in the mouse fetuses.

## Materials and Methods

Following approval from Eastern Mennonite University's Animal Use and Care Committee, Swiss outbred mice were obtained from a national supplier [41], given free access to a diet of Purina rodent chow and water, and housed in a separate room held at 24°C with a 12:12 light: dark cycle for the duration of the experiment. Prior to the start of experimentation, male mice (average 24-26 g body weight) and female mice (average 20-22 g body weight) were allowed to acclimate with their surroundings. A pre-trial group of three control females was run to ensure proper experimental procedure. At the time of the beginning of the experiment both male and female mice were young mature adults averaging 50-60 days of age.

## Experimental Groups and Design

Three experimentation groups were formed with female mice. Control females (CO) received intraperitoneal (IP) saline injections (normal saline 0.03 ml/g per maternal body weight) on gestation days 5 and 7. Binge Alcohol females (BA) received IP injections of alcohol (25% v/v of ethanol in normal saline at 0.03 ml/g per maternal body weight) on gestation days 5 and 7. Alcohol-Anthocyanin females (AA) received IP alcohol injections on days 5 and 7 of gestation (ethanol 0.03 ml/g 25% v/v of ethanol in normal saline per maternal body weight) and subcutaneous-dorsal neck anthocyanin injections on gestation days 0,5,7,12 (anthocyanin, 30 mg/kg per maternal body weight). The anthocyanin injection solution was prepared at a concentration of 5 mg/ml in normal saline from Life Extension Blueberry extract capsules [42]. The concentrations of alcohol and anthocyanin were largely based on prior work in other laboratories [43, 44].

On day 0, cohorts of female mice, representing the three experimental groups, were mated with age-matched males. Gestation day one was determined by the subsequent presence of a vaginal plug. Males were removed 3 days after vaginal plug appearance and rebred with the next cohort of females. To maintain accuracy in food consumption measurements, males were all removed on day four and feeding data measurements began. Throughout the gestation period, food consumption, weight changes and appearances of individual females were recorded.

# DATA COLLECTION AND ANALYSIS

## Necropsy

On gestation day 15, mothers were euthanized with an overdose of ether, their uteruses were excised, and individual fetuses were isolated. After each fetus was removed from its amniotic sac, the fetus was measured from the cranial to caudal end (crown-rump length) using a calipers, weighed to the nearest mg, and photographed, before being placed in either 10% buffered formalin fixative for subsequent histological analysis or frozen for subsequent biochemical analysis.

## Measurements/Stereological Data

Random representative fetuses from each group (N = 12) were used for morphometric and stereological data collection. Morphometric data consisted of determining gross liver and telencephalon area with direct measurements using a Nikon SMZ 74ST microscope and NIS Elements BR 3.2 software. In each fetus the telencephalon, a part of the forebrain area (prosencephalon), and the liver area were determined via specific somatic landmarks. These organ areas were circumscribed and their surface areas estimated in $mm^2$ using the calibrated software program from the image camera. Subsequently, these areas were compared with the total fetus body surface area.

To obtain primary stereological data, a coherent Weibel grid imprinted on an acetate sheet was superimposed on photomicrographs of the fetuses. Following an established protocol, [45, 46] simple point counts, based on the number of Weibel grid points falling on the image of the parameter of interest, e.g., liver vs total body area or telencephalon area vs. total body area, were converted into volume density determinations. Each measured fetus was contained within one counting field of view and represented an “n” of one.

## Statistical Analysis

Means and standard errors were calculated as group statistics for measured parameters: fetal weight, crown-rump length, and gross tissue measurements (organ areas and organ volume density measures). Significant differences

between groups were determined using One-Way ANOVA and Student-Newman-Keuls post-hoc statistical testing ($p < 0.05$) with SPSS 22 software.

## RESULTS AND DISCUSSION

### Maternal Responses

Throughout the experiment pregnant female health and weight were ascertained daily. The maternal body weight of each trial group at the beginning and at the end of the experiment revealed that the CO group averaged 29.5 g at the beginning and finished with an average of 38.4 g. The BA group began with an average of 29.6 g and finished with an average of 36.6 g. The AA group averaged 28.4 at the beginning of the experiment and averaged 37.2 g at the end of the experiment. In summary the pregnancy weight gains were similar for all three experimental groups with an average increase during the first 15 days of 25% (See Table 1). While the CO and AA group mothers trended toward higher body weight gains during their pregnancies than the mothers in the BA group, these values were not statistically different (27% and 28% versus 21% respectively).

**Table 1. Maternal Data: Average Body Weights and Pregnancy Results**

| Experiment Groups | Number of Pregnant Mothers | Fertility Percent | Body Weight Day 1 (g) | Body Weight Day 8 (g) | Body Weight Day 15 (g) | Percent Increase in Body Weight | Total Fetuses | Average Number Fetuses / Mother |
|---|---|---|---|---|---|---|---|---|
| CO | 12 | 80% | 29.5 | 31.4 | 38.4 | 27% | 117 | 9.75 |
| BA | 7 | 58% | 29.6 | 30.5 | 36.6 | 21% | 58 | 8.29 |
| AA | 9 | 75% | 28.4 | 30.6 | 37.2 | 28% | 79 | 8.78 |
| Average | 9.3 | 71% | 29.2 | 30.8 | 37.4 | 25% | | 8.94 |

Groups Definitions: CO = Control: pregnant mothers treated with two saline injections; BA = Binge Alcohol: pregnant mothers treated with two binge alcohol injections; AA = Alcohol-Anthocyanin: pregnant mothers treated with two binge alcohol injections and supplemented with four anthocyanin injections.

Although the project was initiated with mating 15 female mice in the CO group and 12 female mice in the BA and AA groups, the number of resultant pregnant females in the BA and the AA groups was less than in the CO group: CO = 12 pregnancies (80% fertility); BA = 7 pregnancies (58% fertility); AA = 9 pregnancies (75% fertility). While the average number of fetuses per pregnant mother varied slightly in the different groups with an average of 8.94 fetuses/mother, these differences were not statistically significant (See Table 1). Upon conclusion of the project, it was determined that one of the males used was sterile, since each of the females this male mated throughout the project did not produce offspring.

All females receiving binge alcohol injections exhibited similar patterns of behavior following injections that included staggering and losing consciousness within the span of a few minutes. But then later these females revived and resumed normal activity after a period of time. Control females with saline injections did not exhibit these behavioral patterns. Instead they displayed mild agitation following injections.

The average food intake per day by each group generally reflected a steady increase in consumption throughout the period of pregnancy. However food intake did fluctuate as a consequence of alcohol injections (see Figure 1).

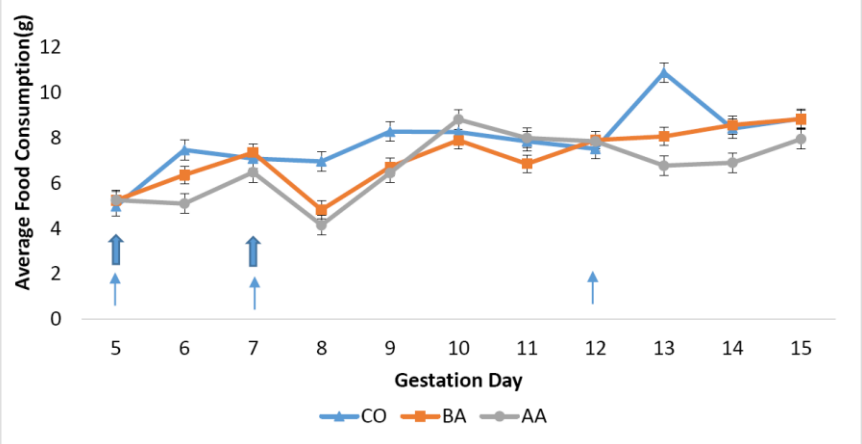


Figure 1. Daily food consumption by experimental group mothers over the course of experiment. Control (CO) saline treated mice (N = 15), Binge Alcohol (BA) treated mice (N = 12), Alcohol–Anthocyanin (AA) treated mice (N = 12). Feeding data began after males were removed from female cages on day 4. Arrows denote days injections were performed (large=ethanol) (narrow = anthocyanin). Values are expressed as means ± standard error.

On day 6 following the day 5 alcohol injections, the BA and AA mothers consumed less food than the CO mothers. More dramatically following the day 7 ethanol injections, the BA and AA mothers' food consumption dropped by almost 30%. In contrast the CO group food consumption remained stable. Food consumption did rebound for both the BA and AA groups in the following days and reached the level of the CO group by day 10 of gestation.

## Fetal Whole Body Responses

Following necropsy, the average weight of the 117 CO fetuses was 196.7 mg (Figure 2) and the average crown to rump length was 11.19 mm (Figure 3). The BA group of 58 fetuses averaged 90.4 mg for fetal weight at time of collection and a length of 9.07 mm. The AA group of 79 fetuses averaged a weight of 161.8 mg and a length of 10.71 mm. Both fetal body weight and crown-rump length were not significantly different between the CO and AA groups. However, significant differences were found between the CO and BA groups as well as between AA and BA groups. Representative samples from each experimental group demonstrating the developmental differences pictorially are illustrated in Figure 4. Strong definition and detail differences in the fetal mice can be more clearly observed in the CO (Figure 4A) and AA (Figure 4C) mice while gross detail was far less distinct in the BA (Figure 4B) mice. Especially in the BA group, the fetuses were frequently ill-formed, but less so in the AA group when compared to the CO group.

In concordance with previous work, perigestational ethanol exposure does retard gross fetal size, [47] however proactive administration of anthocyanins appears to partially neutralize the deficits in fetal size. These results clearly demonstrate that the anthocyanin dosages mitigate the detrimental effects of the alcohol at the very least on a gross fetal body scale.

## Fetal Telencephalon Response

In comparing telencephalon size with the rest of the fetal body (Figure 5), BA fetuses demonstrated a trend of reduced telencephalon size (5%) compared with those of CO (7%) and AA (7%). A similar trend was seen in another study [48] that showed improper telencephalon division and development in ethanol induced mouse embryos.

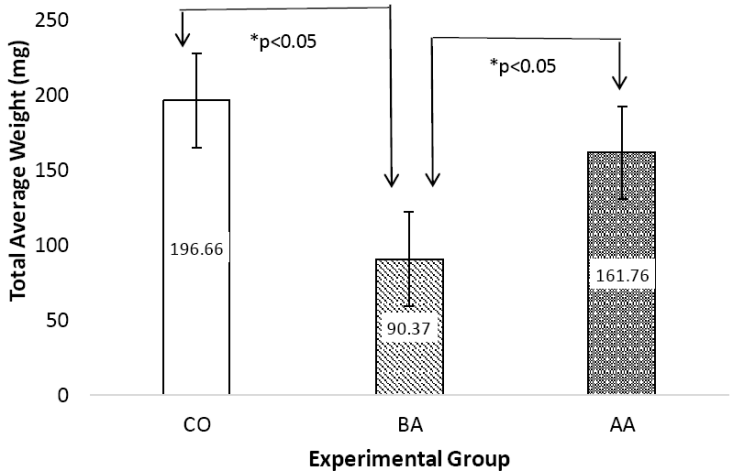


Figure 2. Fetal weights at gestation day 15 across experimental groups N = 254. Control (CO) saline treated fetuses (N = 117), Binge Alcohol (BA) treated fetuses (N = 58), Alcohol-Anthocyanin (AA) treated fetuses (N = 79). Black arrows correspond with statistically significant differing values ($p < 0.05$). Values are expressed as means ±standard error.

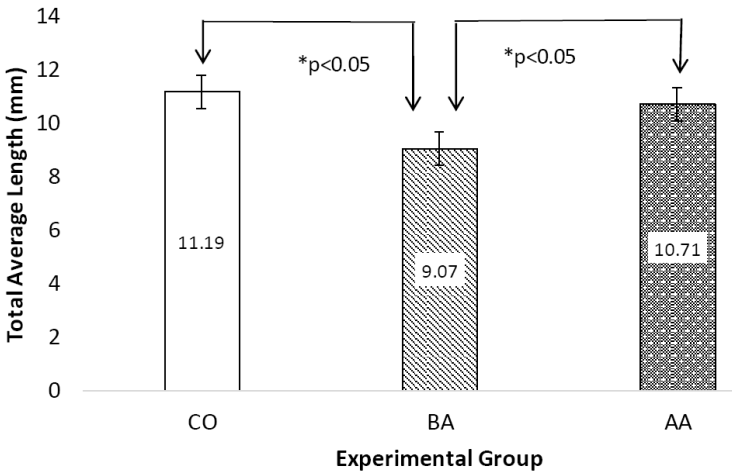


Figure 3. Fetal crown-rump length at gestation day 15 across experimental groups N = 254. Control (CO) saline treated fetuses (N = 117), Binge Alcohol (BA) treated fetuses (N = 58), Alcohol-Anthocyanin (AA) treated fetuses (N = 79). Black arrows correspond with statistically significant different values ($p < 0.05$). Values are expressed as means ± standard error.

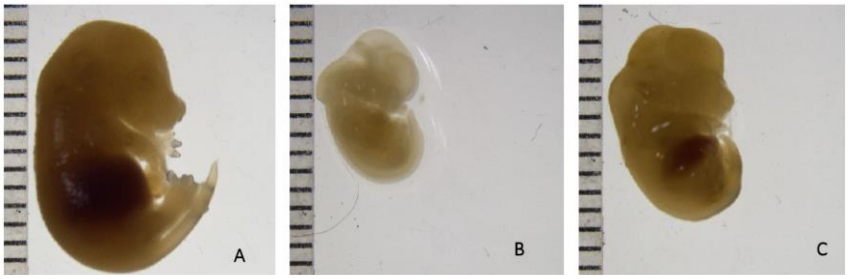


Figure 4. Representative fetal photograph samples at 15 days gestation from each experimental group demonstrating variations on gross body parameters. 4A: Control (CO) saline treated mouse fetus; 4B: Binge Alcohol (BA) treated mouse fetus; 4C: Alcohol–Anthocyanin (AA) treated mouse fetus.

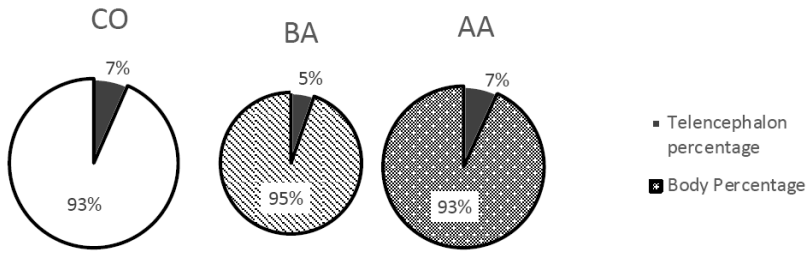


Figure 5. Fetus body composition comparing telencephalon size to relative body size, using a Weibel grid to determine volume density measures. Control (CO) saline treated fetuses (N = 12), Binge Alcohol (BA) treated fetuses (N = 11), Alcohol-Anthocyanin (AA) treated fetuses (N = 11). Values are expressed as means. Circle diameters reflect relative differences in experimental group body sizes.

The reason behind this pattern can be explained by ethanol's ability to impede the regular migration of pluripotent cells, which plays a vital role in the developing central nervous system [49]. In normal brain development, the neural plate acts as a precursor to the developing spinal cord and brain. With ethanol exposure however, the regular developmental processes in BA fetuses may have been hindered, compromising the integrity of the overall size of the brain [50]. The reduction in telencephalon size is not only due to reduced body size but also reduced telencephalon size relative to body size.

Figure 6, which details exact measurements of telencephalon area, demonstrates that AA fetuses have relatively similar telencephalon areas compared with CO fetuses (2.75 versus 3.18 $mm^2$) while BA fetuses (1.47 $mm^2$) have significantly underdeveloped telencephalon areas. The sizeable telencephalon difference between the experimental groups can be attributed to

alcohol-induced oxidative stress which is a major mechanism causing cell death [51]. To explain why telencephalon measurements were similar between AA and CO fetuses, oxidative stress has been shown to decrease upon the administration of cyanidin-3-glucoside, the main anthocyanin compound [52]. Another study supports these protective properties of anthocyanins by showing that anthocyanin administration prevented ethanol-induced neuronal cell death in rat hippocampal cells [53]. The results from our experiment suggest that the ability to maintain the size of the telencephalon in AA fetuses may be attributed to anthocyanin buffering the rate of ethanol-induced neuronal cell death.

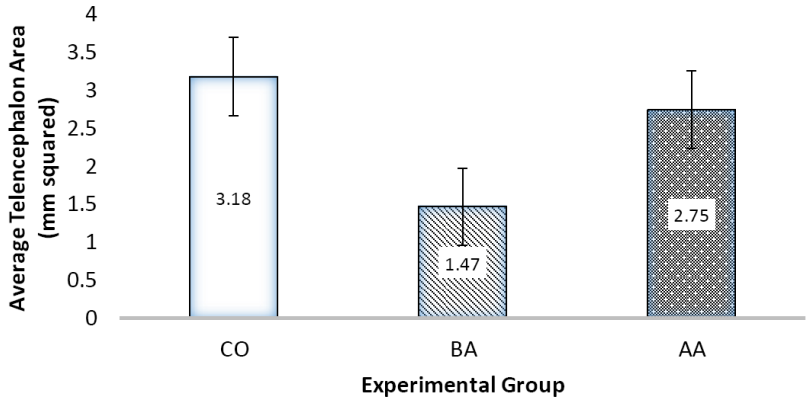


Figure 6. Morphometric telencephalon analysis detailing average absolute telencephalon area in each experimental groups. Control (CO) saline treated fetuses (N = 12), Binge Alcohol (BA) treated fetuses (N = 11), Alcohol-Anthocyanin (AA) treated fetuses (N = 11). Values are expressed as means ± standard errors.

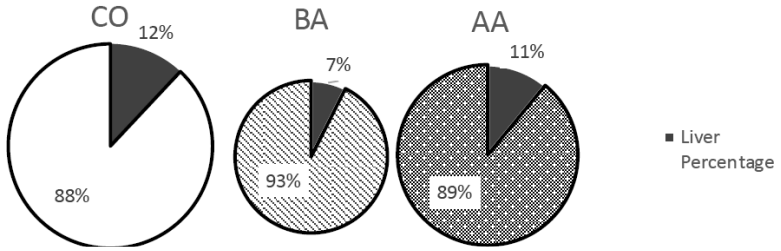


Figure 7. Fetus body composition comparing liver size to relative body size, using a Weibel grid to determine volume density measures. Control (CO) saline treated fetuses (N = 12), Binge Alcohol (BA) treated fetuses (N = 11), Alcohol-Anthocyanin (AA) treated fetuses (N = 11). Values are expressed as means. Circle diameters reflect relative differences in experimental group body sizes.

## Fetal Liver Response

Some of the current research is focused on postnatal liver functioning of mice, which experienced perigestational ethanol exposure. These studies are helpful in showing how binge ethanol exposure can increase hepatic triglycerides and lead to hepatic steatosis through alterations in gene expression [14, 15]. Additionally, in adult models, changes in gene expression and lipid metabolism are central to the effects that acute doses of ethanol prompt in the liver [30].

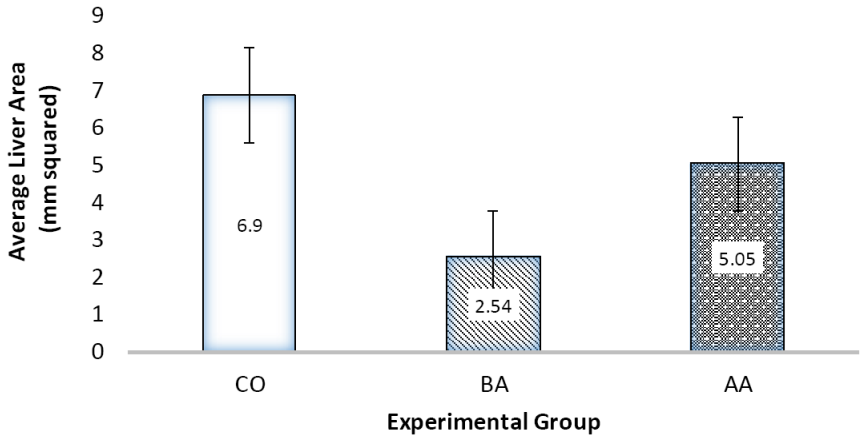


Figure 8. Morphometric liver analysis detailing average absolute liver area in each experimental group. Control (CO) saline treated fetuses (N = 12), Binge Alcohol (BA) treated fetuses (N = 11), Alcohol-Anthocyanin (AA) treated fetuses (N = 11). Values are expressed as means ± standard errors.

In comparison, our results show prenatal fetal reductions of liver size in relationship to relative body size as well as declining absolute liver area due to perigestational ethanol exposure in the BA groups. The introduction of anthocyanins (blueberry extracts) in combination with the ethanol however effectively retains fetal liver size relative to body size as well as absolute liver area. Figure 7 details the relationship between relative liver sizes to body sizes among the experimental groups. Unsurprisingly, BA fetuses show reduced liver size compared to body size (7%), while AA fetuses show retention of liver sizes to body sizes in proportions similar to CO fetuses (11% versus 12% respectively). When morphometric absolute liver area measurements among experimental groups were compared (Figure 8), BA livers were much smaller

(2.54 $mm^2$), while AA fetuses demonstrated retention of gross liver areas similar to that of CO mice (5.05 vs 6.9 $mm^2$). Together Figures 7 and 8 show that BA fetuses not only exhibit reduced gross liver area compared with CO and AA, they also exhibit reduced gross liver size even when their reduced body size is accounted for. This trend is not observed in the AA group, but rather retention of overall absolute liver area as well as liver size in proportion to relative body size is demonstrated when compared with the CO group.

## CONCLUSION

Our research demonstrates that anthocyanin supplementation (in the form of blueberry extracts) given to developing embryos/fetuses in the mouse development system mitigates some of the detrimental effects of concomitant perigestational exposure of alcohol. This mitigating response is seen following two binge alcohol exposures during the early period of gestation when accompanied with four applications of anthocyanin supplementation given before, during, and after the exposure to alcohol.

Initial gross fetal body assessments in comparison to control fetuses show that binge alcohol exposure reduces average fetal body weight by 54% while binge alcohol with anthocyanin supplementation reduces average fetal body weight by 18%. When considering fetal size as determined by crown-rump length, binge alcohol reduces size by 19% and binge alcohol supplemented with anthocyanins reduces size by 4% when compared with control fetuses.

In looking at the size of two organs – telencephalon and liver – a similar outcome is observed. Based on surface area, fetuses in the control group have telencephalon and liver surface areas that represent 7% and 12% respectively of their total body surface area. In contrast binge alcohol fetuses have telencephalon and liver surface areas that represent 5% and 7% respectively of their total body surface area. However, fetuses from the binge alcohol and anthocyanin supplemented group have telencephalon and liver surfaces that represent 7% and 11% respectively of their total body surface area paralleling the control group data. Inspection of these data shows that anthocyanin supplementation has a beneficial effect in reducing the influence of alcohol toxicity.

The specific mechanism for the protective role of anthocyanins against alcohol toxicity in the developing mouse system is not yet determined. Subsequent studies extending this research are currently focusing on

histological and functional parameters of the liver and telencephalon to further elucidate this phenomenon.

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