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INTRODUCTION

Binge drinking (BD) is traditionally defined as a pattern of alcohol consumption that rapidly elevates blood ethanol levels (BELs) to 80 mg/dL or above. It represents an alarming public health concern among adolescents and young adults. However, preclinical models of heavy episodic alcohol consumption frequently overlook female biology, despite growing evidence suggesting that BD may induce sex-specific neurobiological, metabolic and endocrine alterations.

Objective. The investigation aimed to compare sex-differences under two distinct alcohol exposure paradigms: fixed-dose *vs.* variable-dose protocols. BELs, neuroendocrine activation (plasma corticosterone levels, as a marker of the hypothalamic-pituitary-adrenal (HPA) axis activation); and hepatic clearance mechanisms (alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) enzymes) were measured in both experimental designs.

MATERIALS AND METHOD

ALCOHOL ADMINISTRATION

TIME	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
7:00h		EtOH 3 g·kg ⁻¹	EtOH 3 g·kg ⁻¹	EtOH 3 g·kg ⁻¹	EtOH 3 g·kg ⁻¹
15:00h	EtOH 5 g·kg ⁻¹	EtOH 3 g·kg ⁻¹	EtOH 3 g·kg ⁻¹	EtOH 3 g·kg ⁻¹	
23:00h	EtOH 3 g·kg ⁻¹	EtOH 3 g·kg ⁻¹	EtOH 3 g·kg ⁻¹	EtOH 3 g·kg ⁻¹	

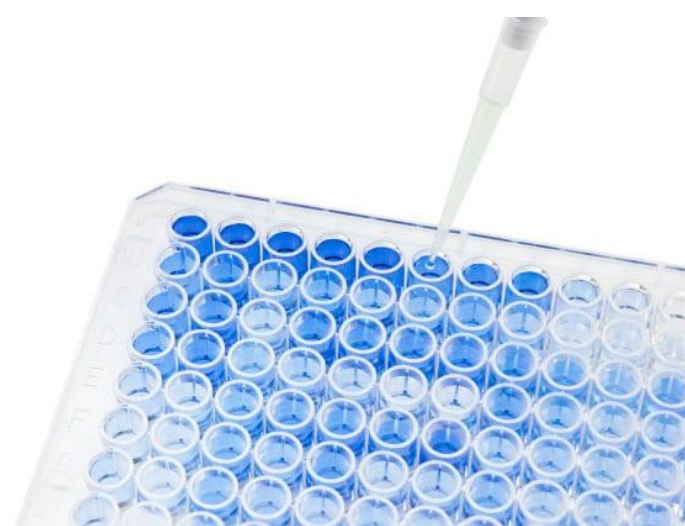
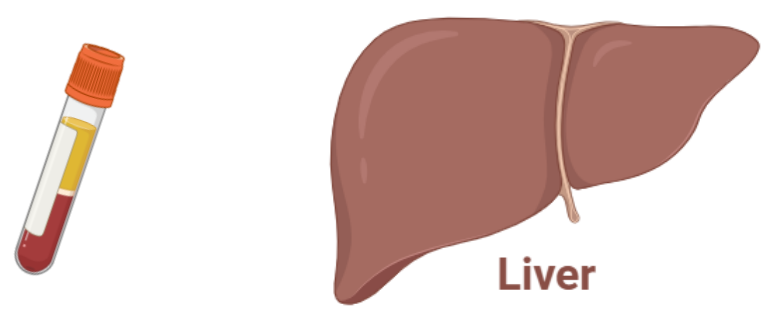
Table 1

Score	Behavioural Indications	EtOH Dose
0	Normal rat	5 g/kg
1	Hyopactive, mild ataxia	4 g/kg
2	Ataxia (elevated abdomen)	3 g/kg
3	Delayed righting réflex Severe ataxia (drags abdomen)	2 g/kg
4	Loss of righting réflex	1 g/kg
5	Loss of eye-blink reflex	0 g/kg

Table 1. Intoxication Scale. Behavioral scale used to determine the level of intoxication during ethanol administration of each animal².

SAMPLES COLLECTION

Plasma and liver were collected



Protein analysis
ELISA assays

STUDY 1

Males and females Wistar rats were exposed to **repeated intragastric binge ethanol administrations** (3 times/day x 4 days) with a maximum dose of 3 g/kg of ethanol 30%, following modified Majchrowick’s binge model¹.

STUDY 2

Males and females Wistar rats were exposed to ethanol binges depending on **behavioural signs of ethanol intoxication** (3 times/day x 4 days; ethanol 30%), following Majchrowick’s Intoxication Scale¹ showed in Table 1.

RESULTS

STUDY 1

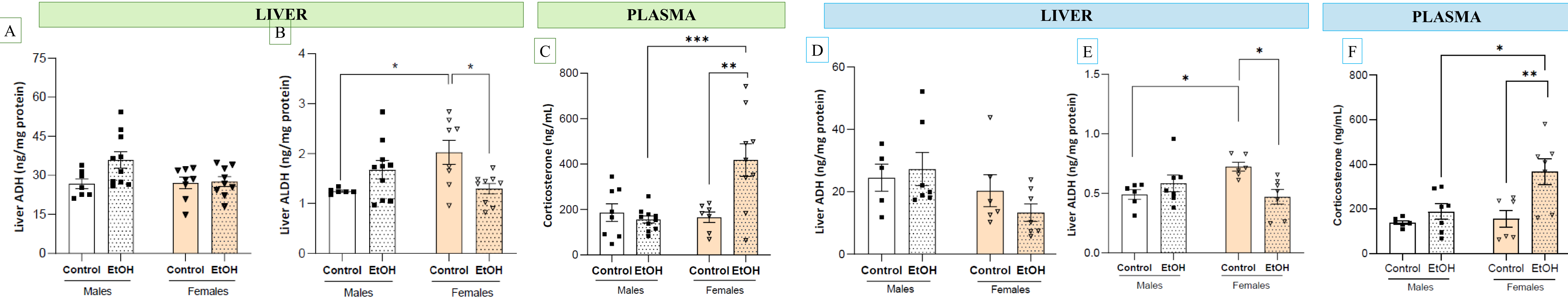
Table 2	MALES		FEMALES		t-test
	Control	EtOH	Control	EtOH	
BELS (mg/dL)	-----	101.52 ±19.61	-----	56.95 ± 17.09*	<i>p</i> * = 0.0491
Accumulative doses (g/kg/day)	-----	9.45 ± 0.105	-----	9.48 ± 0.054	<i>p</i> * = 0.8039

Table 2. BELs and accumulative doses per day in male and female rats in Study 1. Mean daily doses, expressed in g/kg, did not differ significantly between groups (Student’s t-test, *p* > 0.05) but BELs reached 3 hours after the last ethanol administration differed significantly between groups (Student’s t-test, *p* < 0.05), reaching binge levels (> 80 mg/dL) only the males. Data are expressed as mean ± S.E.M. *BELS*=blood ethanol levels; *EtOH*=ethanol group.

STUDY 2

Table 3	MALES		FEMALES		t-test
	Control	EtOH	Control	EtOH	
BELS (mg/dL)	-----	280,1 ± 38,39	-----	212,7 ± 35,01	<i>p</i> = 0,2188 n.s
Accumulative doses (g/kg/day)		9,429 ± 0,1867		10,43 ± 0,3120	<i>p</i> * = 0,0176

Table 3. BELs and accumulative doses per day in male and female rats in Study 2. Due to behavior-guided dose corrections, mean daily doses (g/kg) differ significantly between groups (Student’s t-test, *p* < 0.05). In this case, BELs reached 3 hours after the last ethanol administration were similar (Student’s t-test, *p* > 0.05) and both females and males reached binge levels (> 80 mg/dL). Data are expressed as mean ± S.E.M. *BELS*=blood ethanol levels; *EtOH*=ethanol group.



(A) Expression of liver ADH; **(B)** Expression of liver ALDH; **(C)** Plasma corticosterone levels³ in control and ethanol-treated male and female rats in Study 1. All data are expressed as mean ± S.E.M. Two-way ANOVA followed by Tuckey *post hoc* test: **p* < 0.05. *ADH*=alcohol dehydrogenase; *ALDH*=aldehyde dehydrogenase; *EtOH*=ethanol-treated animals.

(D) Expression of liver ADH; **(E)** Expression of liver ALDH; **(F)** Plasma corticosterone levels in control and ethanol-treated male and female rats in Study 1. All data are expressed as mean ± S.E.M. Two-way ANOVA followed by Tuckey *post hoc* test: **p* < 0.05. *ADH*=alcohol dehydrogenase; *ALDH*=aldehyde dehydrogenase; *EtOH*=ethanol-treated animals.

The results showed that **standard fixed doses** usually used for males **failed to induce binge levels in female subjects**, whereas **behaviour-guided dossing** successfully allowed females to **achieve BELs** in the range of binge **by administration of significantly higher doses of ethanol to females compared with males**. Distinct stress and metabolic profiles emerged between the sexes in both experiments: **ethanol-treated females exhibited higher levels of corticosterone** indicating a state of profound HPA axis reactivity that may correlate with heightened anxiety. Alcohol-treated females also **exhibited a significant reduction in liver ALDH** compared to their respective control group in both studies. This reduction may implicate a potential accumulation of toxic acetaldehyde in females which could exacerbated tissue damage. No significant differences between groups were observed in liver ADH levels.

CONCLUSIONS

The experimental data demonstrated **that applying only male-centric frameworks to preclinical alcohol research inherently limits the translational value** of the studies. Equivalent doses, behavioural outcomes or BELs after alcohol intoxication could mask different internal physiological status, highlighting the critical importance of integrating sex as a primary biological variable.

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