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Ameliorative effects of Bronco-T on formaldehyde-induced biochemical disruptions in the liver and muscle of male albino rats

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Abstract

The objective of this investigation was to assess the potential protective effect of the herbal formulation Bronco-T against deleterious biochemical perturbations induced by formaldehyde in the liver and muscle tissues of male albino rats. The study aims to elucidate how formaldehyde exposure influences the equilibrium of protein, carbohydrate, antioxidants, and oxidative processes within the liver and muscle tissues of albino rats. Additionally, we investigated the direct relationship between observed biochemical alterations and the degree of formaldehyde exposure. In response to formaldehyde-induced abnormalities, administration of Bronco-T demonstrates significant promise. Bronco-T effectively mitigated aberrations in oxidative and antioxidant enzymes, restoring a more balanced state. Furthermore, Bronco-T facilitated improvements in protein and carbohydrate metabolism, playing a pivotal role in addressing the cascade of biochemical changes induced by formaldehyde exposure. It is noteworthy that inhALTion-based exposure to formaldehyde was associated with notable modifications in muscle and liver tissues. Through a comprehensive exploration of these dynamics, our study provides valuable insights into the potential protective role of Bronco-T against formaldehyde-induced biochemical disruptions in liver and muscle tissues, shedding light on its broader implications for health and well-being.

Keywords: *Formaldehyde, toxicity, metabolism, Bronco-T, protective effects*

Formaldehyde (FA), a simple aldehyde composed of carbon, hydrogen, and oxygen, is pervasive in blood, intercellular tissue, and intracellular compartments, acting as a potent electrophile capable of interacting with biological nucleophiles within proteins and DNA (Kamps *et al.,* 2019). While humans naturally produce approximately 44.4 ml of FA daily as part of metabolic processes (Pietzke *et al.,* 2020), exposure to exogenous FA vapours can lead to formaldehyde poisoning, occurring through interactions with sanitised equipment or direct chemical contact

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(Bowerman *et al.,* 2021). Recent studies underscore correlations between impaired FA metabolism and organ dysfunction, bone marrow failure, leukaemia, and liver cancer in animal models (Zhang *et al.,* 2013; Pontel *et al.*, 2015; Dingler *et al.,* 2020).

Despite historical studies suggesting that high-level, lifelong FA exposure did not induce leukemia in rodents (Kerns *et al.,* 1983; Malek *et al.,* 2004), emerging research demonstrates that short-term, low-level FA exposure can lead to brain toxicity in mice (Nielsen *et al.,* 2017; Tesfaye *et al.,* 2021). The impact of FA on leukaemogenic targets such as hematopoietic stem cells (HSC) and progenitor cells (HPC) in the bone marrow (BM) remains an area requiring further investigation. InhALTion of FA vapours may result in lung fluid accumulation and bronchial constriction, presenting as asthma symptoms and bronchial inflammation (Payani *et al.,* 2019; Sholapuri *et al.,* 2020; Owen *et al.,* 1990). Furthermore, FA metabolism primarily occurs in the liver after exposure through various routes, potentially causing hepatotoxicity (Oluwafemi *et al.,* 2020). Accumulated FA has also been associated with osteoporosis and muscle atrophy (Yao *et al.,* 2021). Notably, plant-based medicines enriched with anti-inflammatory or immune-modulating constituents have shown promise in counteracting these effects (Deepak *et al.,* 2020; Sasi *et al.,* 2020; Kocyigit and Guler, 2021; Neenu *et al.,* 2022). Plant-derived remedies are frequently employed to mitigate the negative outcomes of chemical toxicity (Kotnis *et al.,* 2004; Singh *et al.,* 2016).

In this context, our study focuses on evaluating the efficacy of polyhedral formulation (Bronco-T) in alleviating the detrimental impacts of FA-induced toxicity, specifically in liver and muscle tissues. Our primary objective was to investigate the protective potential of the herbal supplement Bronco-T against harmful biochemical irregularities triggered by formaldehyde in the liver and muscle tissues of male albino rats. Recent studies supporting the protective role of herbal formulations similar to Bronco-T against formaldehyde-induced toxicity further underscore the importance of investigating such interventions for potential health benefits.

Materials and methods

Animals

All albino rats were handled in compliance with the Institutional Ethical Committee's guidelines for the care and use of laboratory animals of Sri Venkateswara University (Resolution No. 10(i)/a/CPSEA/IAEC/SVU/ZOOL/MB/Dt.08-07-2012), India. Healthy male Albino rats, 30 in number, weighing between 180 – 250 g procured from LABVIVO services PVT LTD, Bangalore were used for this study. They were housed in each polycarbonate cage under standard laboratory conditions at a room temperature of 24 ± 25°C and humidity 45-64% with 12h light/dark cycle and fed with standard diet obtained from Sri Venkateshwara Enterprises Bangalore, India. Water was supplied through plastic bottle provided with nipples.

Experimental design

Fig. 1 depicts the study's overall experimental design in a nutshell. The animals were divided into five groups, each group containing 6 rats. Group1 served as control rats and group 2 to 5 were experimental rats. All the treatment procedure took place for 21 days. After treatment animals were sacrificed and liver and muscle tissue removed for biochemical assessment. To assess Bronco-T's effects alone (Group 3), it was administered to the animal through oral gavage.

Sample collection and analysis

After the exposure period, animals were sacrificed, and liver and muscle tissues were promptly collected and promptly processed for enzymatic analysis. Subsequently, these tissues were preserved in a 10% formalin solution to maintain structural integrity and inhibit decay, thereby ensuring the preservation of tissue architecture for precise biochemical assays.

Estimation of antioxidant enzymes

Five per cent homogenate (w/v) of liver and muscle tissues were prepared simultaneously in 50 mM iced up phosphate

(a)

S. No.	Category	Treatment
1.	Group-1 (Control)	Without any treatment fed solely a commercial diet
2.	Group-2	Treated with FA (exposed to 40% FA for one hour daily)
3.	Group-3	Treated with Bronco-T (0.2 mg/kg bw-through oral gavage)
4.	Group-4	Treated with FA (exposed to 40% FA for one hour daily) + Treated with Bronco-T
5.	Group-5	Treated with FA (exposed to 40% FA for one hour daily) + Treated with 0.15 mg/kg/dose salbutamol (through oral gavage)

(b)

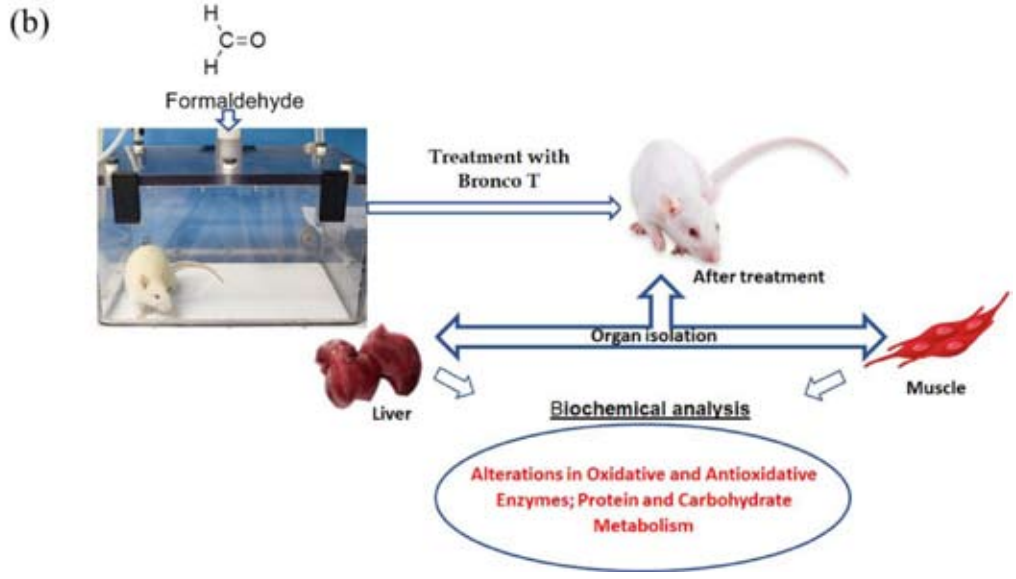

Fig. 1. (a): Experimental grouping of animal experiments; (b) Schematic representation of the study's comprehensive experimental framework.

buffer (pH 7.0) containing 0.1 mM EDTA. Centrifugation was carried out at 10,000 rpm for 10 min at 4°C. The supernatant was separated and used for the enzyme assays of superoxide dismutase (SOD) and catalase (CAT). The calculation of SOD activity involved measuring the optical density at 480 nm for 4 min using a Hitachi U-2000 spectrophotometer, following the method outlined by Misra and Fridovich (1972). The enzyme activity was expressed as units per milligram of protein (U/mg protein). One unit of superoxide dismutase was completely inhibited in the presence of 20 micromolar sodium cyanide (NaCN). The catalase activity was calculated from decrease in the absorbance of reaction mixture at 240 nm in UV -spectrophotometer according to the method of Aebi (1984) and Ramachandraiahgari *et al.* (2012). Here, CAT activity was expressed as moles of H_2O_2 degraded per minute per milligram of protein (mol H_2O_2/min/mg protein). For the estimation of glutathione peroxidase (GPx) activity, we followed the method of Carlberg and Mannervik (1985). The unit of GPx activity was expressed as micromoles of NADPH oxidized per minute. Similarly, for the estimation of lipid peroxidase activity, we followed the method outlined by Ohkawa*et al.* (1979). The lipid peroxidase activity was expressed as micromoles of malondialdehyde formed per minute

Isolation of mitochondria

The procedure for isolating the mitochondria from rat liver and muscle tissue

was carried out exactly as reported by Forner *et al.* (2006). The isolated mitochondria were metabolically active, tightly linked, and largely free of extracellular impurities. The preparations could be kept for several weeks when frozen in liquid nitrogen without any discernible loss of enzyme activity.

Estimation of Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) activities

The activity of AST in the mitochondrial fraction of rat liver and muscle was assayed using the method adopted by Ueda *et al.* (1967) and the enzyme activity was expressed as µmoles of pyruvate formed per milligram of protein per minute. Alanine aminotransferase (ALT) activity in the mitochondrial fraction of rat liver and muscle was assayed at 545nm in a UV- spectrophotometer by the method of Reitman and Frankel (1957), as described by Bergmeyer and Bruns (1965). The enzyme activity was expressed as µ moles of pyruvate formed / mg protein /min.

Estimation of redox enzyme activities

Prepared in an ice-cold 0.25 M sucrose solution, homogenates of the liver and muscle tissues (10% w/v) were centrifuged at 1000g for 15 minutes at 4°C. The supernatant fraction was used for enzyme assays of lactate dehydrogenase (LDH), succinate dehydrogenase (SDH), and glucose-6-phosphate dehydrogenase (G-6-PDH). The activity of LDH was determined at 495 nm in a spectrophotometer by measuring the formazone using the protocol described by Nachlas *et al.,* (1960) as advised by Prameelamma and Swami (1975) with little changes and expressed in moles of formazone formed/mg protein/min. Further, Nachlas *et al.* (1960) also developed a method to check the specific activity of SDH by the measuring the absorbance at 495 nm in a spectrophotometer as enzymes utilize FAD and INT to form formazone and this was expressed in micromoles of formazone formed / mg protein / min. Glucose-6-phosphate dehydrogenase activity was calculated as per the method adapted from Lohr and Waller (1965), as modified by Mastanaiah *et al.* (1978) and expressed in µ moles of formazone formed / mg protein / min.

Estimation of protein metabolism

Protein metabolism estimation involved determining total protein concentration in treated liver and muscle tissues following the method by Lowry *et al.* (1951) with BSA as the standard. Liver and muscle tissues (5 g) were homogenised in 10 mL of double-distilled water for soluble and structural protein estimation. The liver homogenate underwent centrifugation at 2500 rpm for 15 minutes, with the residue used for estimating structural proteins. The supernatant was mixed with an equal amount of 10% TCA for protein precipitation. After standing for 30 minutes at room temperature, the mixture was centrifuged at 2500 rpm for 30 minutes, and the residue represented the water-soluble protein fraction, dissolved in 1 mL of 1 N sodium hydroxide. The resulting solution had 4 mL of alkaline copper reagent and 0.4 mL of Folin-phenol reagent added, and the colour was measured at 600 nm using a spectrophotometer. Protein content was expressed as mg/g wet weight. Free amino acids from treated rat liver and muscle were extracted and quantified using the protocol by Schurr *et al*. (1950) with standard curves generated from a composite reference for large routine amino acid studies. The levels of free amino acids were measured in treated rat liver and muscle tissue using these standard curves.

Estimation of carbohydrate metabolism

The total carbohydrate content in the liver and muscle tissues of control, FA, B-T, FA + B-T, and FA + S treated albino rats was estimated using the method of Carrol *et al.* (1956). Lactic acid in the liver and muscle tissues of both control and treated albino rats was estimated by the method of Barker and and Summerson (1941), modified by Huckabee *et al.* (1961). The pyruvic acid content in the homogenate of both control and treated albino rats' liver and muscle was estimated using the method of Friedemann and Haugen (1942). The glucose content in the liver and muscle tissues of both control and treated albino rats was estimated by the method of Kemp and Van Heijningen (1954).

Table 1. Effects of formaldehyde, salbutamol, and Bronco-T on liver antioxidant and other metabolic enzymes (Means ±S.E. in each row, followed by the same letter are not significantly different (P≤0.05) from each other according to DMR test)

Liver	Group-I (C)	Group-II (FA)	Group-III (B-T)	Group-IV (FA + B-T)	Group –V (FA + S)
SOD	9.44±0.9^{c}	5.21±0.52^{a}	9.67±0.54^{d}	8.984±0.71^{b}	9.35±0.94^{c}
CAT	12.85±1.08^{c}	4.98±0.46^{a}	12.94±0.23^{c}	12.15±1.01^{b}	12.67±1.12^{c}
GR	1.62±0.17^{b}	0.55±0.02^{a}	1.71±0.08^{b}	1.57±0.09^{b}	1.59±0.12^{b}
LPO	12.49±1.12^{b}	22.64±2.14^{c}	12.52±0.54^{b}	11.78±1.33^{a}	11.58±1.18^{a}
AST	14.08±0.11^{b}	22.19±2.09^{d}	12.11±0.35^{a}	16.90±0.21^{c}	16.25±0.14^{c}
ALT	2.94±0.23^{a}	6.23±0.16^{c}	2.32±0.10^{a}	3.96±0.16^{b}	3.65±0.18^{b}

Statistical analysis

All the experimental data given in the results were means of triplicates Duncan's new Multiple range (DMR) test was used to find significant difference (P<0.05) between values of each sampling.

Results and discussion

The significant reduction in SOD and CAT activities in Group-II (Formaldehyde exposure) suggested an imbalance in antioxidative defences, leading to heightened oxidative stress in the liver (Table 1). This is indicative of formaldehyde-induced damage, as these enzymes play crucial roles in neutralising reactive oxygen species (ROS). The notable decrease in GR activity in Group-II further supported the oxidative stress induced by formaldehyde. GR is essential for maintaining the reduced state of glutathione, a critical antioxidant, and its reduction indicates compromised antioxidative capacity. The marked increase in LPO in Group-II reflects substantial damage to liver cell membranes due to oxidative stress. Elevated LPO is a sign of lipid degradation and oxidative damage, further highlighting the adverse effects of formaldehyde exposure on liver tissues. The elevated levels of AST and ALT in Group-II indicate liver impairment, suggesting that formaldehyde exposure has a detrimental impact on liver function. The trends toward restoration of antioxidant enzyme activities (SOD, CAT, GR) and reduction in LPO in treated group suggest a potential hepatoprotective effect of Bronco-T. It may counteract the oxidative damage induced by formaldehyde, supporting liver health. Similar trends in antioxidatint enzyme activities indicate a potential hepatoprotective effect of Salbutamol when combined with formaldehyde. This suggests a protective role of Salbutamol against formaldehyde-induced oxidative stress in the liver.

Similar to the liver, formaldehyde exposure in Group-II led to a significant reduction in SOD and CAT activities in muscle tissues, indicating heightened oxidative stress and potential damage (Table 2). The decrease in GR activity further supports oxidative damage in muscle tissues due to formaldehyde exposure. The increase in LPO reflects oxidative damage to muscle cell membranes, highlighting the adverse effects of formaldehyde on muscle tissues. Elevated levels of AST and ALT in Group-II suggest potential impairment of muscle function due to formaldehyde exposure. Similar to the liver, B-T or salbutamol treated groups displayed trends toward the restoration of antioxidant enzyme activities (SOD, CAT, GR) and reduction in LPO, suggesting potential protective effects of Bronco-T and Salbutamol against formaldehyde-induced oxidative damage in muscle tissues. The improvements in AST and ALT levels in these groups further support the potential of Bronco-T and Salbutamol to mitigate the adverse effects of formaldehyde exposure on muscle function. The findings emphasize the significant impact of formaldehyde on liver and muscle tissues, inducing oxidative stress and impairing enzymatic activities. Bronco-T and Salbutamol show promise as potential interventions to counteract these effects, highlighting the

importance of exploring pharmaceutical strategies for protecting against environmental pollutants' detrimental impact on liver and muscle function.

While there are very few records of literature on the protective role of Bronco-T against formaldehyde-induced biochemical disruptions in the liver and muscle of any organism (Payani *et al.,* 2019; Sholapur *et al.,* 2020), some studies suggest that specific plant extracts with antioxidant properties may offer protection against formaldehyde-induced damage (Sayyar *et al.,* 2018; Paul *et al.,* 2020; Oluwafemi *et al.,* 2020). These extracts could enhance the activity of antioxidant enzymes, scavenge free radicals, and mitigate the impact of formaldehyde on cellular structures. The results of the present study significantly bolster the protective efficacy of Bronco-T against formaldehyde-induced toxicity, with positive antioxidant enzyme outcomes contributing to the growing evidence supporting its pivotal role in alleviating adverse effects associated with formaldehyde exposure.

Redox enzyme activities

The outcomes delineated in Tables 3 and 4 underscore the profound impact of formaldehyde exposure on redox enzyme activities in both liver and muscle tissues of male albino rats. In Group-II, the pronounced reduction in succinate dehydrogenase (SDH), lactate dehydrogenase (LDH), and glucose-6-phosphate dehydrogenase (G-6-PDH) activities signifies a disturbance in the delicate balance of redox homeostasis. The substantial decrease in SDH activity implies compromised mitochondrial function, potentially disrupting the electron transport chain and contributing to impaired cellular respiration. Concurrently, the diminished LDH activity suggests a disruption in anaerobic glycolysis, indicating a compromised energy metabolism in response to formaldehyde exposure. The reduction in G-6-PDH activity further accentuates the vulnerability of cellular antioxidant defences, as this enzyme is instrumental in maintaining the pool of reduced glutathione and mitigating oxidative stress.

Conversely, Groups III, IV, and V demonstrate encouraging trends towards the restoration of redox enzyme activities, indicating a potential protective effect of Bronco-T and Salbutamol against the deleterious consequences of formaldehyde exposure. Particularly noteworthy is the observed improvement in SDH, LDH, and G-6-PDH activities in both liver and muscle tissues. These findings strongly suggest that Bronco-T may act as a therapeutic agent, mitigating the oxidative stress induced by formaldehyde and potentially restoring cellular functions. The results not only shed light on the redox-related disruptions caused by formaldehyde but also highlight the promising role of polyherbal interventions, emphasizing the need for further investigations into the mechanisms by which Bronco-T and Salbutamol exert their protective effects. These insights underscore the potential of polyherbal medicine in attenuating the adverse effects of environmental toxins on cellular redox status and open avenues for

Table 2. Effects of formaldehyde, salbutamol, and Bronco-T on muscle antioxidant enzymes (Means ±S.E. in each row, followed by the same letter are not significantly different (P≤0.05) from each other according to DMR test).

Muscle	Group-I (C)	Group-II (FA)	Group-III (B-T)	Group-IV (FA + B-T)	Group –V (FA + S)
SOD	7.04 ± 0.52^{b}	3.81 ± 0.32^{a}	7.78 ± 0.51^{c}	6.96 ± 0.61^{b}	7.01 ± 0.6^{b}
CAT	9.15 ± 0.8^{c}	4.58 ± 0.41^{a}	10.15 ± 0.92^{d}	8.55 ± 0.73^{b}	9.57 ± 0.9^{c}
GR	0.84 ± 0.02^{c}	0.44 ± 0.012^{a}	0.85 ± 0.023^{c}	0.68 ± 0.09^{b}	0.82 ± 0.02^{c}
LPO	10.29 ± 1.02^{a}	15.83 ± 2.04^{c}	11.5 ± 1.12^{b}	10.48 ± 1.23^{a}	10.85 ± 1.01^{a}
AST	5.94 ± 0.51^{a}	8.16 ± 0.69^{c}	5.14 ± 0.43^{a}	6.18 ± 0.50^{b}	6.03 ± 0.54^{b}
ALT	0.96 ± 0.09^{a}	1.55 ± 0.062^{b}	0.81 ± 0.02^{a}	0.95 ± 0.088^{a}	0.91 ± 0.098^{a}

Table 3. Effects of formaldehyde, salbutamol, and Bronco-T on liver redox enzymes (Means ±S.E. in each row, followed by the same letter are not significantly different (P≤0.05) from each other according to DMR test)

Liver	Group-I (C)	Group-II (FA)	Group-III (B-T)	Group-IV (FA + B-T)	Group –V (FA + S)
SDH	10.96±1.14^{b}	2.85± 0.08^{a}	9.85±0.34^{b}	8.72±0.15^{b}	9.11±0.72^{b}
LDH	6.23±0.14^{d}	1.54±0.11^{a}	5.96±0.25^{d}	3.72±0.16^{b}	4.63±0.12^{c}
G-6-PDH	4.38±0.41^{c}	0.91±0.032^{a}	4.12±0.18^{c}	2.96±0.11^{b}	3.24±0.22^{b}

Table 4. Effects of formaldehyde, salbutamol, and Bronco-T on muscle redox enzymes (Means ±S.E. in each row, followed by the same letter are not significantly different (P≤0.05) from each other according to DMR test).

Muscle	Group-I (C)	Group-II (FA)	Group-III (B-T)	Group-IV (FA + B-T)	Group –V (FA + S)
SDH	8.21±0.17	2.74±0.12^{a}	8.85±0.21	5.45±0.72^{b}	6.25±0.32^{c}
LDH	2.51±0.22^{c}	1.13±0.11^{a}	2.73±0.14^{c}	2.11±0.21^{b}	2.45±0.04^{c}
G-6-PDH	3.62±0.19^{c}	1.62±0.17^{a}	3.82±0.35^{c}	2.94±0.32^{b}	3.11±0.21^{b}

the development of targeted interventions in oxidative stress-related pathologies. To the best of our knowledge, no reports currently exist regarding the effectiveness of Bronco-T in mitigating redox enzymes in any organ against formaldehyde-induced biochemical disruptions. The available literature on this specific aspect is limited. However, there is existing research on the potential protective role of certain plant extracts against the deleterious effects of formaldehyde (Paul *et al.,* 2020; Ezekiel, 2021). The findings of the present study demonstrate a notable improvement compared to prior research, thereby enhancing and corroborating the protective efficacy of Bronco-T against formaldehyde-induced toxicity. The observed enhancements in redox enzyme activity in this investigation contribute to a growing body of evidence suggesting that Bronco-T may serve as a significant mitigator of the deleterious effects linked with formaldehyde exposure.

Protein metabolism

The exhaustive analysis of protein metabolism in liver and muscle tissues, as detailed in Tables 5 and 6, unveils the complex ramifications of formaldehyde exposure and the prospective ameliorative effects of Bronco-T and Salbutamol on protein homeostasis. The notable decline in total proteins observed in the liver tissue of formaldehyde-exposed rats (Group-II) suggests a disturbance in hepatic protein synthesis. This decrease could be ascribed to formaldehyde-induced modifications in protein turnover and degradation mechanisms.

The decline in structural proteins in Group-II suggests a compromise in the integrity of liver tissue, possibly due to formaldehyde-induced protein denaturation or degradation of structural components. A decrease in soluble proteins further emphasizes the impact of formaldehyde on the liver's functional protein pool. The decline in soluble proteins may contribute to impaired cellular processes and functions. The diminished levels of free amino acids in formaldehyde-exposed rats highlight a disruption in amino acid availability for protein synthesis and other cellular processes. Conversely, Groups III (Bronco-T), IV (Formaldehyde + Bronco-T), and V (Formaldehyde + Salbutamol) exhibit trends toward the restoration of protein levels. The elevation observed in total proteins, structural proteins, soluble proteins, and free amino acids implies a possible protective influence of Bronco-T and Salbutamol against formaldehyde-induced protein depletion in liver tissues. These results underscore the importance of polyherbal and pharmacological interventions

Table 5. Effects of formaldehyde, salbutamol, and Bronco-T on protein metabolism in liver tissue (Means ±S.E. in each row, followed by the same letter are not significantly different (P≤0.05) from each other according to DMR test)

Liver	Group-I (C)	Group-II (FA)	Group-III(B-T)	Group-IV (FA + B-T)	Group –V (FA + S)
Total Proteins	164.45±12.13^{b}	150.05±10.04^{a}	175.01± 14.29^{c}	158.12±7.21^{b}	161.12±11.00^{b}
Structural Proteins	79.12±7.05^{b}	71.99±5.08^{a}	91.09±6.06^{c}	73.27±6.14^{a}	75.89±5.52^{a}
Soluble Proteins	85.74±8.05^{b}	76.54±7.55^{a}	96.21±6.08^{c}	79.41±5.22^{a}	81.92±7.75^{a}
Free Amino acids	9.41±0.82^{d}	7.43±0.75^{a}	11.30±1.4^{d}	8.64±0.32^{b}	9.09±0.03^{c}

Table 6. Effects of formaldehyde, salbutamol, and Bronco-T on protein metabolism in muscle tissue (Means ±S.E. in each row, followed by the same letter are not significantly different (P≤0.05) from each other according to DMR test)

Muscle	Group-I (C)	Group-II (FA)	Group-III (B-T)	Group-IV (FA + B-T)	Group –V (FA + S)
Total Proteins	174.45±12.13^{c}	152.05±0.04^{a}	178.23±9.32^{c}	161.12±0.02^{b}	163.12±0.03^{b}
Structural Proteins	75.12±7.05^{b}	61.99±0.08^{a}	76.11±5.14^{b}	71.89±7.52^{b}	74.60±7.11^{b}
Soluble Proteins	86.97±6.05^{ab}	72.54±5.55^{a}	89.32±5.34^{ab}	82.92±6.7^{b}	84.01±6.15^{b}
Free Amino acids	4.98±1.01^{b}	3.45±0.85^{a}	4.81±0.08^{c}	4.42±0.14^{b}	4.52±0.2^{b}

in alleviating formaldehyde-induced disruptions in liver protein metabolism.

The reduction in total proteins in formaldehyde-exposed rats (Group-II) implies a disturbance in muscle protein homeostasis. Formaldehyde may lead to altered rates of protein synthesis and degradation, contributing to decreased total protein levels (Table 6). The decline in structural proteins suggests potential damage to muscle architecture in response to formaldehyde exposure. This may include degradation of contractile proteins, impacting muscle function. The decrease in soluble proteins further accentuates the disruption in functional protein pools within muscle tissues, potentially impacting various cellular processes. The diminished levels of free amino acids in formaldehyde-exposed rats indicate impaired availability of amino acids for muscle protein synthesis and maintenance. In contrast, the administration of Bronco-T (Group III), either alone or in combination with formaldehyde (Group IV), and Salbutamol (Group V) demonstrates trends toward the restoration of protein levels in muscle tissues. This suggests a potential protective role of Bronco-T and Salbutamol against formaldehyde-induced alterations in muscle protein metabolism. The findings underscore the potential therapeutic efficacy of these interventions in ameliorating the adverse effects of formaldehyde on protein homeostasis in both liver and muscle tissues. Further exploration of the underlying mechanisms is crucial for understanding the full scope of these interventions in mitigating formaldehyde-induced disruptions in protein metabolism.

As of current knowledge, there is a notable absence of reports documenting the effectiveness of Bronco-T in mitigating

Table 7: Effects of formaldehyde, salbutamol, and Bronco-T on carbohydrate metabolism in liver tissue (Means ±S.E. in each row, followed by the same letter are not significantly different (P≤0.05) from each other according to DMR test).

Liver	Group-I (C)	Group-II (FA)	Group-III (B-T)	Group-IV (FA + B-T)	Group –V (FA + S)
Total Carbohydrates	52.54±6.10^{cd}	28.32±2.13^{a}	52.97±6.11^{cd}	49.56±4.05^{c}	45.23±1.36^{b}
Lactic Acid	24.54±2.05^{a}	34.25±2.8^{c}	25.14±2.06^{a}	32.73±3.01^{b}	31.41±1.63^{b}
Pyruvic Acid	29.52±2.09^{c}	9.12±0.81^{a}	30.47±2.19^{c}	23.4±2.02^{b}	21.72±4.14^{b}
Glucose	7.08±0.71^{d}	5.11±0.18^{a}	7.19±0.07^{d}	6.38±0.14^{c}	6.11±0.21^{c}

protein metabolism disruptions in any organ exposed to formaldehyde-induced biochemical disturbances (Sholapuri *et al.*, 2020). However, a limited number of studies have investigated the potential mitigating effects of certain plant extracts in this regard (Yalcin *et al.,* 2015; Abdel Hamid *et al.,* 2022). It is important to note that the available literature on Bronco-T specifically addressing protein metabolism in the context of formaldehyde-induced disruptions is scarce. In contrast to the existing knowledge gap, the findings from the current study provide affirmative evidence regarding Bronco-T's protective capacity in mitigating protein metabolism disturbances induced by formaldehyde toxicity. This revelation marks a significant contribution to the field, suggesting that Bronco-T may indeed play a role in preserving protein homeostasis amidst formaldehyde-induced challenges.

Carbohydrate metabolism

The intricate interplay of carbohydrate metabolism in both liver and muscle tissues, as revealed in Tables 7 and 8, provides valuable insights into the disruptive effects of formaldehyde exposure and the potential ameliorative impact of Bronco-T and Salbutamol on glucose, lactic acid, and pyruvic acid metabolism. The significant decrease in total carbohydrates in formaldehyde-exposed rats (Group-II) suggests a disruption in hepatic glucose storage or synthesis. This reduction may indicate an impairment in glycogen synthesis or an increased demand for glucose in response to formaldehyde-induced stress. The heightened lactic acid levels observed in Group-II suggest an augmentation in anaerobic glycolysis, possibly signaling a transition towards lactate production due to formaldehyde exposure. The concurrent decrease in LDH levels and increase in lactate may signify diminished tissue damage alongside intensified glycolytic activity or hypoxic conditions, indicating dynamic metabolic alterations. This alteration may signify an adaptive response to changes in cellular energy demands. The decrease in pyruvic acid levels in formaldehyde-exposed rats suggests a potential inhibition of glycolysis or altered conversion of pyruvate to acetyl-CoA. This may be indicative of disruptions in the tricarboxylic acid (TCA) cycle and mitochondrial function.

The reduction in glucose levels further emphasizes the impairment of glucose homeostasis in formaldehyde-exposed rats,

Table 8. Effects of formaldehyde, salbutamol, and Bronco-T on carbohydrate metabolism in muscle tissue (Means ±S.E. in each row, followed by the same letter are not significantly different (P≤0.05) from each other according to DMR test).

Muscle	Group-I (C)	Group-II (FA)	Group-III (B-T)	Group-IV (FA + B-T)	Group –V (FA + S)
Total Carbohydrates	78.54±7.12^{c}	38.32±3.1^{a}	79.97±7.14^{c}	69.76±4.05^{b}	71.34±3.45^{b}
Lactic Acid	30.54±3.02^{a}	58.25±5.24^{c}	31.24±3.06^{a}	38.73±3.25^{b}	36.54±2.36^{b}
Pyruvic Acid	31.54±3.09^{c}	12.12±1.11^{a}	32.46±3.12^{c}	27.43±2.09^{b}	26.56±3.11^{b}
Glucose	10.08±0.11^{d}	5.07±0.51^{a}	10.42±0.12^{e}	8.32±0.4^{b}	9.08±0.4^{c}

potentially indicating increased glucose utilization or compromised gluconeogenesis. Groups III, IV, and V treated with Bronco-T and Salbutamol, show improvements in carbohydrate metabolism compared to Group-II. The observed increase in total carbohydrates, coupled with the normalisation of lactic acid, pyruvic acid, and glucose levels, suggests a potential protective effect of Bronco-T and Salbutamol against formaldehyde-induced disturbances in liver carbohydrate metabolism. These findings underscore the significance of these interventions in restoring hepatic glucose homeostasis.

The significant decrease in total carbohydrates in formaldehyde-exposed rats (Group-II) implies a disruption in muscle glycogen storage or synthesis (Table 8). This reduction may indicate an increased demand for glucose or impaired glycogen utilization in response to formaldehyde-induced stress. The elevated levels of lactic acid in Group-II indicate an increase in anaerobic glycolysis, possibly as a compensatory mechanism for altered energy demands in muscle tissues due to formaldehyde exposure. The decrease in pyruvic acid levels in formaldehyde-exposed rats suggests potential alterations in glycolysis or impaired conversion of pyruvate to acetyl-CoA in muscle tissues. The reduction in glucose levels further emphasizes the impairment of glucose homeostasis in formaldehyde-exposed rats, potentially indicating increased glucose utilization or compromised gluconeogenesis in muscle tissues. Groups III, IV, and V, treated with Bronco-T and Salbutamol, show improvements in carbohydrate metabolism compared to Group-II. The observed increase in total carbohydrates, coupled with the normalization of lactic acid, pyruvic acid, and glucose levels, suggests a potential protective effect of Bronco-T and Salbutamol against formaldehyde-induced disturbances in muscle carbohydrate metabolism. These findings underscore the significance of these interventions in restoring muscle glucose homeostasis.

To date, very few or no reports have documented the efficacy of Bronco-T in mitigating disruptions in carbohydrate metabolism in any organ exposed to formaldehyde-induced biochemical disturbances. Existing literature on this specific aspect is limited, with a few studies investigating the potential mitigating effects of certain plant extracts (Klein *et al*., 2022; Manan *et al.,* 2022). It is noteworthy that the available information on Bronco-T specifically addressing carbohydrate metabolism in the context of formaldehyde-induced disruptions is scarce. However, the present study contributes valuable insights by affirming that Bronco-T possesses protective abilities in mitigating carbohydrate metabolism disturbances induced by formaldehyde toxicity. The results from this research shed light on the intricate alterations in carbohydrate metabolism within both liver and muscle tissues in response to formaldehyde exposure. Moreover, the study underscores the potential therapeutic significance of Bronco-T and Salbutamol in mitigating the adverse impact of formaldehyde on glucose, lactic acid, and pyruvic acid metabolism. These findings emphasize the necessity for further exploration of these interventions in the context of environmental toxin-induced metabolic disturbances. The recognition of the protective effects of Bronco-T and Salbutamol against formaldehyde-induced disruptions in carbohydrate metabolism provides a foundation for future research aimed at elucidating the underlying mechanisms and optimizing these interventions for potential clinical applications in mitigating metabolic imbalances associated with environmental toxin exposure.

Conclusion

Our study demonstrates that exposure to FA significantly impacts protein, carbohydrate, antioxidant, and oxidative balance in the liver and muscle tissues of albino rats, directly correlating with FA vapor exposure. Treatment with Bronco-T following FA exposure effectively restored abnormalities in oxidative and antioxidant enzyme activity, while enhancing protein and carbohydrate metabolism, indicative of its protective properties. Biochemical changes observed in liver and muscle tissues following FA exposure, whether through inhalation, injection, or other routes, encompass a spectrum of alterations ranging from macroscopic changes in size and color to microscopic and biochemical manifestations. Nonetheless, it is imperative to

acknowledge that further research is warranted to comprehensively elucidate the mechanisms of action and determine optimal dosages of Bronco-T for mitigating formaldehyde toxicity.

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Conflict of interest

The authors do not have any conflict of interest.

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