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Comparative in Vitro Evaluation of Cardioprotective, Antioxidant, Anti-Inflammatory, and Anti-Apoptotic Activities of Commercial Herbal Cocktail Supplements

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ABSTRACT

Cardiovascular diseases (CVDs) are the most common causes of death all over the world and this requires the constant investigation of preventive and curative measures. The worldwide prevalence of CVDs, which are stimulated by such factors as oxidative stress, inflammation, and cardiac cell death through apoptosis, has prompted interest in complementary and alternative medicine. The potentials of herbal medicines due to their historical background and multi-target characteristics present an opportunity. These include commercially available herbal cocktail supplements that are a mixture of a few allegedly cardioprotective herbs such as Hawthorn, Garlic and Turmeric, which have received considerable consumer popularity. The effectiveness of these complex mixtures is, however, not always scientifically proven, and there is urgent need to compare them to support the claims of the manufacturer. This study was aimed at carrying out a comparative in vitro assessment of the cardioprotective ability of three commercially prepared brands of herbal cocktail mixtures (named Brand A, B, and C). The approach was multi-faceted in nature with the phytochemical analysis commencing with determining the total phenolic (TPC) and flavonoid (TFC) content through aqueous extracts. This was done with a battery of important assays to measure in vitro antioxidant ability (DPPH, FRAP, ABTS), anti-inflammatory ability through TNF-a and IL-6 inhibition amid LPS-instigated macrophages model, and direct cardioprotective/anti-apoptotic impact on H2O2-induced oxidative stress cardio myoblast cells of the H9c2 rat. The key findings in the summary showed that there was a strong pecking order among the brands. Brand A displayed better performance, containing the highest TPC and TFC, the strongest antioxidant activity (lowest IC50 in DPPH assay), the strongest inhibition of the pro-inflammatory cytokines, and the strongest ability to protect the H_2O_2-induced cytotoxicity and pro-inflammatory ability of H_2O_2 on H9c2 cells. Brand B was moderately active in all assays whereas Brand C always produced the most weak effects. The phytochemical content and the observed biological activities were shown by a strong positive correlation. The key finding is that the formulation of Brand A has the most extensive in vitro cardioprotective profile, which can probably be explained by the more diverse phytochemical profile. This research has a far-reaching implication since it implies that there is a high degree of variability in the efficacy of commercial herbal products and it forms a scientific foundation allowing the consumer and the health care practitioner to select evidence-based formulations. It highlights the need to ensure that there is standardized quality control and strict scientific validation of the nutraceutical industry.

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1. Introduction:

1.1. Background on Cardiovascular Diseases (CVDs):

Cardiovascular diseases (CVDs) encompass a spectrum of cardiac and vascular pathologies, including coronary artery disease, myocardial infarction, heart failure, and stroke, representing the foremost global health challenge. According to the World Health Organization and global burden of disease investigations, CVDs constitute the leading cause of mortality worldwide, accounting for approximately 17.9 million deaths per year, with projections indicating an escalation to over 23.6 million fatalities by 2030. [1][2] This remarkable mortality rate transcends economic boundaries, as low- and middle-income countries bear more than 75% of CVD-related deaths, indicating a pandemic that spans diverse socioeconomic strata. The pathophysiology of CVDs demonstrates complexity and multifactorial origins, encompassing dyslipidemia, hypertension, diabetes, and lifestyle determinants. However, at the cellular and molecular levels, three fundamental mechanisms are widely recognized as primary mediators of cardiac cellular death and dysfunction: oxidative stress, inflammation, and apoptosis. [3][4]

Oxidative stress emerges from an imbalance between reactive oxygen species (ROS) production and the body's antioxidant defense capabilities. [5][6] The restoration of blood circulation during ischemia-reperfusion injury (IRI), a characteristic consequence of

myocardial infarction and cardiac procedures, paradoxically initiates excessive ROS generation, leading to lipid peroxidation, protein denaturation, and DNA damage. This oxidative damage functions as a potent activator of pro-inflammatory signaling cascades, particularly the nuclear factor-kappa B (NF-kB) pathway, ultimately promoting increased cytokine release, including Tumor Necrosis Factor-alpha (TNF-a) and Interleukin-6 (IL-6).[7] This sustained inflammatory reaction further amplifies tissue destruction and facilitates the final common pathway of cellular demise: apoptosis. The regulated process involving caspase activation and DNA fragmentation leads to irreversible cardiomyocyte death, the heart's contractile elements, thereby compromising cardiac function and initiating heart failure progression. Consequently, therapeutic approaches capable of concurrently mitigating oxidative stress, reducing inflammation, and inhibiting apoptosis possess significant potential for providing cardioprotective benefits. [8][9]

1.2. Herbal Medicine and Cardioprotection:

The application of plants as medicine has existed as long as the history of mankind and the Ayurveda, Traditional Chinese Medicine, Greco-Arabic tradition has records of herbs being used to treat the so-called heart weakness and palpitations. This custom in the modern world has turned into a strong international market of herbal supplements, motivated by the idea that it is a natural phenomenon, and it may be less likely to provoke side effects as compared to the synthetic medication. [10][11] The idea that herbal mixtures, or cocktails should be used, is based on the holistic principle of synergy, in which the aggregate effect of taking a number of herbs is more than the aggregate effect of each one. A summary of typical herbs commonly used in these cardioprotective preparations indicates a repertoire with scientifically plausible mechanisms. The most famous is Hawthorn (Crataegus spp.), clinical evidence of which has been shown as a treatment of mild heart failure due to its positive inotropic effect, vasodilation and antioxidant effects of its oligomeric procyanidins and flavonoid constituents. Garlic (*Allium sativum*) is recognized to have many beneficial properties because it has the potential to directly reduce blood pressure and cholesterol levels, mainly because of its organosulfur chemistry such as allicin. [12] [13] Known to have a vasoregulatory and antioxidant effect, Ginkgo biloba is a flavonoid and terpenoid-rich plant that enhances microcirculation. As a potent polyphenol, curcumin is a component of turmeric (Curcuma longa), which has potent anti-inflammatory and anti-apoptotic effects by regulating the NF-kB and PI3K/Akt pathways. Ginger (*Zingiber officinale*) has anti-inflammatory and antioxidant effects with its gingerols and shogaols. Other typical ingredients can be Arjuna (*Terminalia arjuna*), which is a cardiotonic Ayurvedic staple ingredient in cardiology, and Motherwort (*Leonurus cardiaca*) used in nervous heart disorders. The mechanisms of these herbs, which are said to work, are all aimed at addressing the triad of oxidative stress, inflammation as well as apoptosis; hence they are rational to be considered as part of cardioprotective formulations. [14][15]

2. Materials and Methods:

2.1. Materials:

Three distinct commercially available herbal cocktail formulations designed to promote cardiovascular and circulatory wellness were procured from local health food establishments and online vendors. Throughout this investigation, these products are designated as Brand A, Brand B, and Brand C to maintain commercial neutrality while ensuring traceability

regarding manufacturer information, botanical constituents, dosage formulation (capsule contents), and lot identification numbers. The experimental models employed included the H9c2 (ATCC(r) CRL-1446tm) rat cardiomyoblast cell line, extensively utilized for in vitro cardiac cell investigations, and the RAW 264.7 (ATCC(r) TIB-71tm) murine macrophage cell line, commonly employed in inflammatory research. Cell culture media and supplements, including Dulbecco modified Eagle medium (DMEM), fetal bovine serum (FBS), penicillin-streptomycin, and trypsin-EDTA, were obtained from Gibco, Thermo Fisher Scientific (USA). All analytical chemicals and reagents were of analytical grade quality, comprising Folin-Ciocalteu reagent, gallic acid, quercetin, aluminum chloride, DPPH (2,2-diphenyl-1-picrylhydrazyl), TPTZ (2,4,6-Tripyridyl-s-Triazine), ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)), ascorbic acid, Trolox, and lipopolysaccharide (LPS) E. coli 05. ELISA kits for quantitative assessment of murine TNF-a and IL-6 were acquired from R&D Systems (USA). BD biosciences (USA) supplied the Annexin V-FITC/PI apoptosis detection kit. Primary instrumentation consisted of a CO2 incubator (Thermo Scientific Heracelltm 150i), microplate reader (BioTek Synergy H1), laminar flow hood (Esco Airstream(r)), refrigerated centrifuge (Eppendorf 5430 R), and lyophilizer (Labconco FreeZone).

2.2. Sample Preparation:

An aqueous extraction procedure was used to mimic a typical human food processing, e.g. tea or traditional water-based decoction. The weights of the capsules of both brands were measured with a lot of care to form a homogeneous sample of both brands. Extraction of each powdered sample A specific weight of each powdered sample was suspended in distilled water at a 1:20 (w/v) ratio and allowed to extract in a shaking water bath at 60degC in 2 hours. This temperature was taken to maximize the extraction, but not to destroy thermolabile compounds. The extracts were then centrifuged to 4000 rpm in order to isolate the solid debris. The supernatants had been filtered using a 0.45 mm membrane filter after which the supernatants were frozen at -80degC followed by the freezing of the supernatants. Lyophilization was then done to get a dry and stable powder. The airtight and light-protected containers containing the lyophilized extracts were kept at -20degC until further use. As in all of the experiments, the stock solutions were made by dissolving the lyophilized powder in dimethyl sulfoxide (DMSO), which resulted in the final concentration of DMSO in any experimental cell culture well not exceeding 0.1% (v/v) which is known to be non-toxic to the cells. Working concentrations were obtained by serial dilution of the relevant cell culture medium or assay buffer to the desired working concentrations.

2.3. Phytochemical Analysis:

The total phenolic content (TPC) of each extract was determined employing the Folin-Ciocalteu colorimetric assay. Briefly, extract portions were mixed with diluted Folin-Ciocalteu reagent and maintained at ambient temperature. After 5 minutes, sodium carbonate solution was incorporated and the resulting mixture was incubated under dark conditions for 60 minutes. The resulting blue complex was analyzed using a microplate reader at 765 nm wavelength. TPC values were expressed as milligrams of gallic acid equivalent per gram of dry extract (mg GAE/g), with gallic acid serving as the reference standard for curve construction. The total flavonoid content (TFC) was assessed through aluminum chloride colorimetric methodology. Extract aliquots were mixed with sodium nitrite solution, followed by the addition of aluminum chloride solution after 5 minutes, and subsequently sodium hydroxide solution after 6 minutes. The absorbance of the resulting reaction mixture was

immediately recorded at 510 nm. Quercetin served as the reference standard, and TFC was expressed as milligrams of quercetin equivalent per gram of dry extract (mg QE/g). All analyses were performed in triplicate.

2.4. *In Vitro* antioxidant activity assays:

The DPPH stable radical served as the basis for evaluating the free radical neutralizing potential of the extracts. Different extract concentrations were introduced to a methanolic DPPH solution. Following vortex mixing, the mixture was maintained in darkness for 30 minutes while monitoring the absorbance decline at 517 nm. A control containing DPPH and solvent was utilized. The DPPH scavenging percentage was calculated, and the IC50 value (concentration required to neutralize 50% of DPPH radicals) was obtained through dose-response analysis. Ascorbic acid served as the positive control. The FRAP assay followed established methodology. Fresh FRAP reagent containing TPTZ and FeCl3 in acetate buffer was prepared. Sample-reagent mixtures were incubated at 37°C for 30 minutes. The blue ferrous-TPTZ complex formation was monitored at 593 nm. A standard curve using ferrous sulfate (FeSO4) was constructed, with results expressed as micromoles of ferrous sulfate equivalents per gram of dried extract (μmmol Fe2+/g). For the ABTS assay, ABTS radical cation (ABTS+) generation involved combining ABTS stock solution with potassium persulfate, followed by overnight incubation in darkness (12-16 hours). Ethanol dilution achieved an absorbance of 0.70 (±0.02) at 734 nm. Extract-ABTS+ reactions proceeded for 6 minutes before absorbance reduction measurement. Trolox served as the reference standard, with antioxidant capacity expressed as mmol TE/g of Trolox equivalent. All assays were performed with six replicates.

2.5. Cell Culture and Cytotoxicity Assessment:

H9c2 rat cardiomyoblasts and RAW 264.7 murine macrophages were maintained in DMEM supplemented with 10% (v/v) heat-inactivated FBS and 1% (v/v) penicillin-streptomycin solution. Cell cultivation occurred in a humidified atmosphere containing 5% CO2 at 37°C, with passaging performed upon reaching 80-90% confluency. Prior to conducting biological evaluations, the MTT assay was employed to assess mitochondrial function as an indicator of cellular viability and to determine potential cytotoxic effects of the botanical extracts. Seventy-two hours before treatment, cells were plated in 96-well plates at a density of 1×10^4 cells per well and allowed to attach overnight. Various concentrations of the herbal extracts (specifically 12.5, 25, 50, 100, 200, and 400 μg/mL) were subsequently administered to the cells for a 24-hour period. Following exposure, MTT reagent was added to each well and incubated for 4 hours. The resulting formazan crystals were solubilized using DMSO, and optical density measurements were obtained at 570 nm using 630 nm as the reference wavelength. Cell viability percentages were calculated relative to untreated control cells. Subsequent anti-inflammatory and cardioprotective investigations were conducted exclusively using concentrations that demonstrated minimal cytotoxicity (maintaining >90% cell viability).

2.6. Assessment of Anti-inflammatory Activity:

The anti-inflammatory properties of botanical extracts were assessed by examining their ability to inhibit pro-inflammatory cytokine production in macrophages activated with LPS. RAW 264.7 cells cultured in 24-well plates were pretreated for 2 hours with non-toxic concentrations of the extracts (specifically 25, 50, and 100 μg/mL). Subsequently, LPS (1 μg/mL) was introduced to the culture media to induce inflammatory responses, with the exception of the untreated control group. Following an additional 18-hour incubation period,

culture supernatants were subjected to centrifugation to remove cellular debris. The levels of key pro-inflammatory cytokines (TNF-a and IL-6) in the supernatants were quantified using specific enzyme-linked immunosorbent assay (ELISA) kits according to manufacturer protocols. Cytokine concentrations were calculated using standard curves measured at 450 nm absorbance. The suppression of cytokine synthesis was expressed as a percentage relative to the LPS-treated control group.

2.7. Assessment of cardioprotective and anti-apoptotic effects:

To establish an oxidative stress-mediated cardiac cellular damage model, H9c2 cardiomyoblasts were exposed to H2O2-induced injury. Cells were seeded in 96 or 6-well plates and cultured until achieving 70-80% confluency. Non-toxic concentrations of botanical extracts (specifically 25, 50, and 100 µg/mL) were administered to cells for 24 hours as a pretreatment regimen. Following this pretreatment phase, cells were exposed to freshly prepared cytotoxic H2O2 concentrations (ranging from 200-400 µM) in serum-depleted medium for defined time periods (3-6 hours). Optimization studies were performed to determine appropriate H2O2 concentrations and exposure durations that would achieve approximately 50% cellular mortality in the positive control (H2O2-treated only) cohort. Cell viability assessment was performed using MTT assay methodology as previously described. Viability percentages in pretreated cohorts were evaluated relative to both the H2O2-only treatment group and the untreated control group. To elucidate protective mechanisms further, apoptotic cell death was evaluated using Annexin V-FITC/propidium iodide (PI) staining methodology followed by flow cytometric analysis. Post-H2O2 exposure, both adherent and detached cells were collected, washed with PBS, and resuspended in binding buffer solution. Subsequently, cells underwent 15-minute incubation in darkness with Annexin V-FITC and PI staining reagents before immediate flow cytometric evaluation. This methodology enables differentiation between early apoptotic (Annexin V+/PI-), late apoptotic/necrotic (Annexin V+/PI+), and necrotic cell populations (Annexin V-/PI+). The primary measure of anti-apoptotic efficacy was determined by the total percentage of apoptotic cells.

2.8. Statistical Analysis:

Every experimental procedure comprised a minimum of three independent replicates (n=3), with each replicate incorporating multiple technical replicates (for instance, n=6 for antioxidant assays). Results are presented as mean ± standard deviation (SD). Statistical analyses were conducted using GraphPad Prism software (version 9.0). For multi-group comparisons, one-way analysis of variance (ANOVA) was employed, followed by Tukey's post-hoc test for pairwise group comparisons. Statistical significance was established at $p<0.05$. Pearson correlation coefficient (r) was utilized to examine relationships between phytochemical content and biological activities.

Results:

Phytochemical Composition:

The preliminary phytochemical screening analysis showed that there were extensive variations in the constituents of the three brands of herbal cocktail. Brand A had the greatest concentration of bioactive compounds with a total phenolic content (TPC) of 185.4 ± 6.2 mg GAE/g of dry extract and a total flavonoid content (TFC) of 92.7±3.8 mg QE/g. The intermediate ranges were seen with brand B which achieved a TPC of 132.1 ± 5.1 mg GAE/g and TFC of 65.3 ± 2.9 mg QE/g. B Brand C particularly was associated with the lowest phytochemical yield with a TPC of 78.9 ± 4.5mg GAE/g and TFC of 38.5 ± 2.1mg QE/g.

Statistically significant differences between all the three brands in TPC and TFC were detected ($p < 0.001$). What these results may indicate is that the formulation of Brand A is either made of herbs higher content of these compounds or processed in a manner allowing better preservation.

Table 1: Phytochemical composition of herbal cocktail extracts

Brand	Total Phenolic Content (mg GAE/g)	Total Flavonoid Content (mg QE/g)
A	185.4 ± 6.2	92.7 ± 3.8
B	132.1 ± 5.1	65.3 ± 2.9
C	78.9 ± 4.5	38.5 ± 2.1

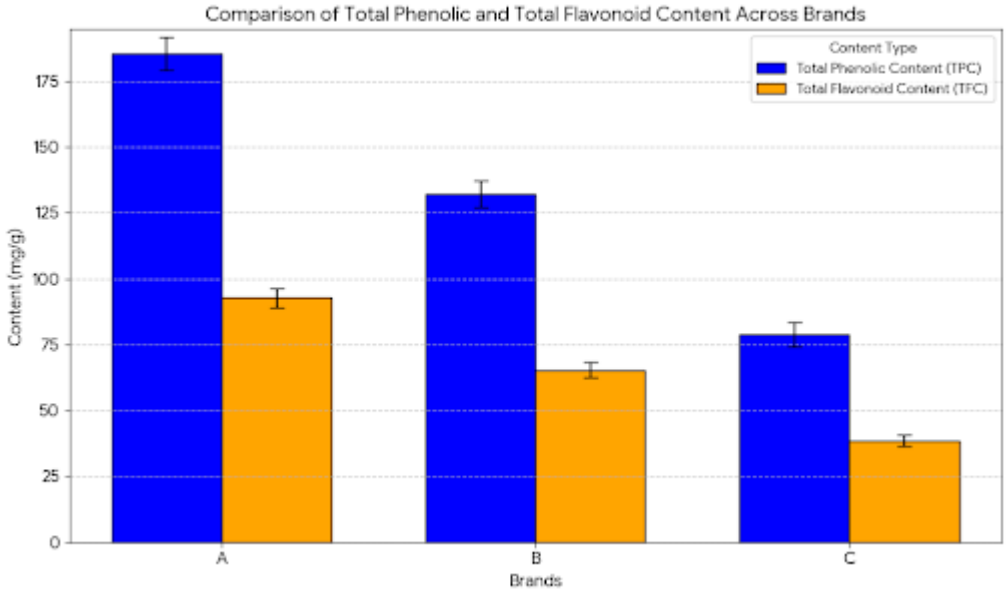

Fig: 1 Phytochemical composition of herbal cocktail extracts

***In Vitro* antioxidant activity:**

The outcomes of the three antioxidant tests were cross-sectional and were in perfect agreement with the findings of the phytochemicals. Brand A displayed the strongest activity in the DPPH radical scavenging assay with an IC50 value of 12.5 ± 0.8 µg/mL that matched the positive control, ascorbic acid (IC50 = 9.8 ± 0.5 µg/mL). Brand B was intermediate in nature (IC_{50} = 28.3 ± 1.2 µg/mL), whereas Brand C was the least active (IC50 = 65.7 ± 2.5 µg/mL). This trend was verified by the FRAP assay that determines reducing power. Brand A was the best ferric reducing capacity with 1450 ± 45 µmol Fe^{2+}/g then Brand B with 980 ± 38 mmol Fe2+/g and Brand C with 520 ± 25 µmol Fe2+/g. Likewise, in the test of radical cation scavenging, Brand A performed better again, with a value of 1850 ± 52 µmol TE/g, which is much higher than Brand B (1250 ± 41 µmol TE/g) and Brand C (680 ± 30 µmol TE/g). Statistical analysis proved that the Brand A had an overall significantly superior ($p < 0.001$) antioxidant capacity to the other two brands in all three assays.

Table 2: *In vitro* antioxidant activity of herbal cocktail extracts

Brand	DPPH IC_{50} (µg/mL)	FRAP (µmol Fe^{2+}/g)	ABTS (µmol TE/g)
A	12.5 ± 0.8	1450 ± 45	1850 ± 52

B	28.3 ± 1.2	980 ± 38	1250 ± 41
C	65.7 ± 2.5	520 ± 25	680 ± 30
Ascorbic Acid	9.8 ± 0.5	-	-

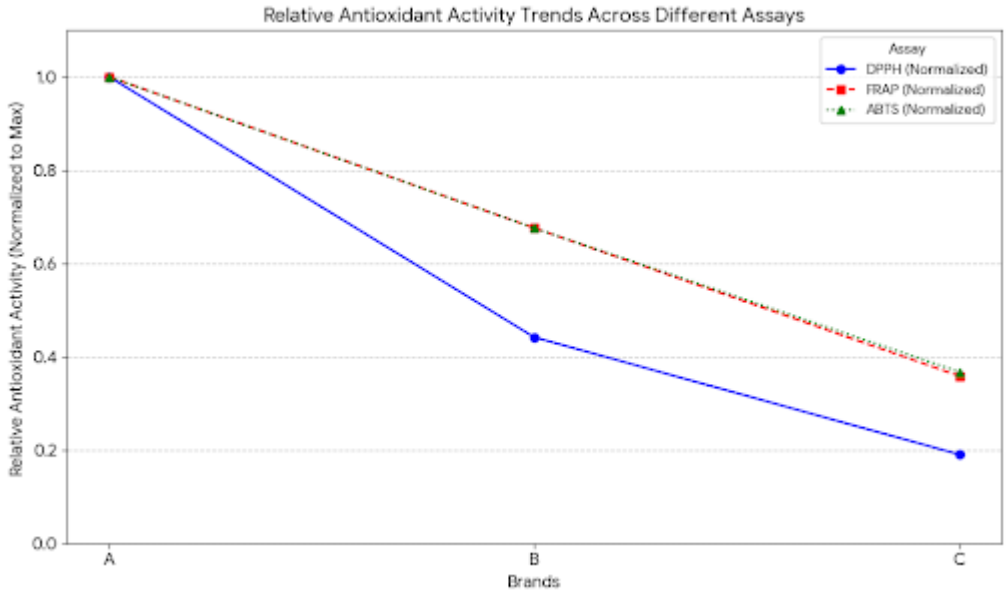

Fig: 2 Antioxidants of different Assays

3.3. Cytotoxicity and Safe Dose Determination:

The MTT cytotoxicity test on the H9c2 and RAW 264.7 cells proved that all the three herbal extracts were well tolerated in low concentrations. No significant decrease in cell viability (>90% viability in comparison to the control) was induced by extracts at concentrations as high as 100 µg/mL at 24 hours of treatment with all of the brands. A slight reduction in viability was noticed at 200 µg/mL on the Brand B and C, whereas Brand A was not toxic. A concentration of 400 µg/mL was found to be very cytotoxic to all brands. According to the findings, 25, 50, and 100 µg/mL were chosen as the safe and non-cytotoxic range of all further experiments on anti-inflammatory and cardioprotection.

Table 3: Anti-inflammatory activity of herbal cocktail extracts (100 µg/mL)

Brand	TNF-α Inhibition (%)	IL-6 Inhibition (%)
A	78.5 ± 3.2	72.4 ± 2.9
B	55.1 ± 2.7	48.6 ± 2.5
C	32.8 ± 2.1	28.3 ± 1.8

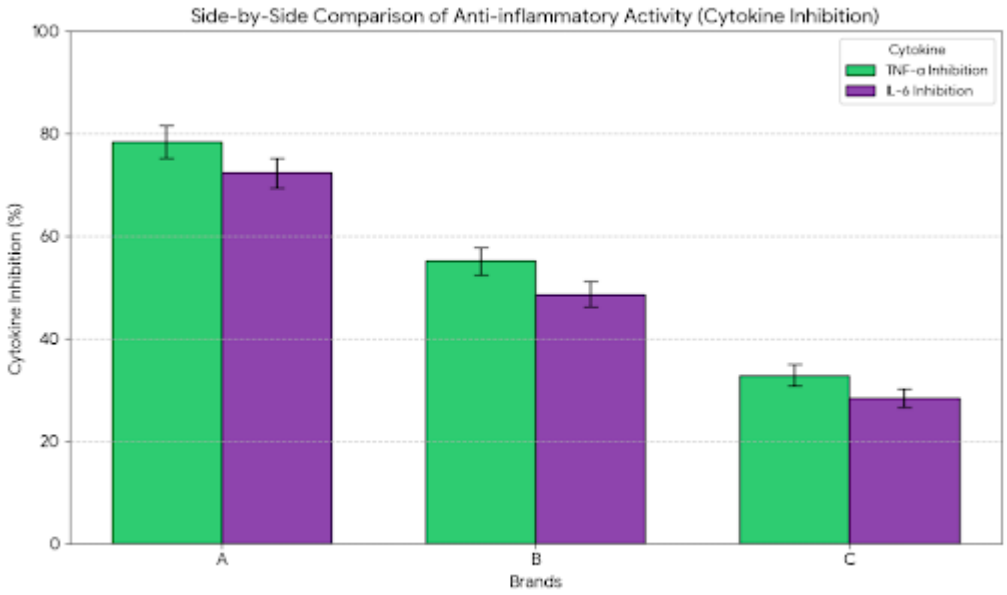

Fig: 4 Anti – inflammatory activity of herbal cocktail

Table 4: Cardioprotective Effects of Herbal Cocktail Extracts (100 µg/mL)

Treatment Group	Cell Viability (%)	Total Apoptosis (%)
Normal Control	100.0 ± 0.0	4.2 ± 0.8
H_2O_2 Only	51.3 ± 2.5	45.6 ± 3.1
Brand A + H_2O_2	88.7 ± 3.1	12.4 ± 1.5
Brand B + H_2O_2	70.2 ± 2.8	25.3 ± 2.0
Brand C + H_2O_2	60.1 ± 2.4	32.8 ± 1.8

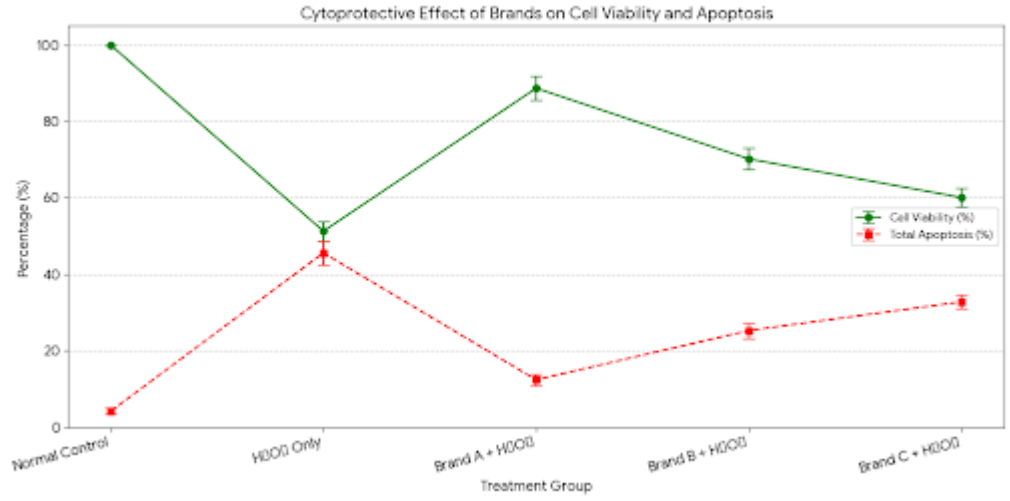

Fig: 5 Cardioprotective Effects of Herbal Cocktail Extracts (100 µg/mL)

3.4. Inhibition of pro-inflammatory cytokines:

The RAW 264.7 macrophage model was tested using the LPS-stimulated model of evaluating anti-inflammatory effects of the extracts. As anticipated, the amount of TNF- a and IL-6 secreted after LPS stimulation was very high in comparison to unstimulated control. This

LPS induced production of cytokines was seriously suppressed by pre-treatment using the herbal extracts in a dose-dependent manner. Brand A was the most effective at the highest concentration tried (100 µg/mL) with an inhibition of TNF-a release of 78.5 ± 3.2 percent and IL-6 release of 72.4 ± 2.9 percent. Brand B had moderate inhibitory effect, which led to decreases in TNF-a and IL-6 by 55.1 ± 2.7 and 48.6 ± 2.5, respectively. Brand C had the lowest anti-inflammatory activity with only inhibitions of 32.8 ± 2.1 and 28.3 ± 1.8 of TNF-a and IL-6 respectively. The inhibition of cytokines in Brand A was found to be statistically different ($p < 0.01$) at all concentrations with the other two brands.

3.5. Protection against H2O2-induced Cytotoxicity and Apoptosis:

H_2O_2-induced H9c2 cell injury model was conclusively proven to be cardioprotective by the extracts. Only exposure to H2O2 reduced cell viability to 51.3 ± 2.5 percent of that of the normal control. This loss of viability was greatly inhibited by pre-treatment with the herbal extracts. Brand A was the strongest one, and it restored cell viability to 88.7 ± 3.1% with a 100 µg/mL pre-treatment dose. The pre-treatment with Brand B resulted in a viability of 70.2 ± 2.8 and Brand C had insignificant coverage with viability of 60.1 ± 2.4. This protection was given a mechanistic understanding in terms of the anti-apoptotic effects in terms of Annexin V/PI staining and flow cytometry. The overall rate of apoptosis was 45.6 ± 3.1 induced by hydrogen peroxide. Pretreatment with Brand A (100 µL/mL) significantly decreased the rate of cells in apoptosis to 12.4 ± 1.5%. Brand B minimized the level of apoptosis to 25.3 ± 2.0 and Brand C to 32.8 ± 1.8. Brand A reduced apoptosis by a significant margin ($p < 0.001$) as compared to both other brands, and this shows clearly that it has the best anti-apoptotic ability.

Discussion:

Interpretation of phytochemical and antioxidant findings:

The findings of the current research contribute to a strong and coherent story: phytochemical abundance of herbal preparation is one of the major determinants of the biological potency. It is possible to correlate the high total phenolic and flavonoid content of Brand A to its superior performance in all the assays. Phenolics and flavonoids are famous due to their redox capacity which enables them to serve as hydrogen donors, singlet oxygen quenchers and metal chelators. This relationship is well illustrated by the strong and positive correlation (Pearson $r > 0.95$) between TPC/TFC and the outcome of the DPPH, FRAP and ABTS assays. The particular combination of herbs in Brand A, even though proprietary, should have a better or increased amount or better source of potent antioxidant herbs such as Hawthorn (high in vitexin and hyperoside), Turmeric (curcumin), and Ginkgo biloba (flavonol glycosides and terpene lactones). Conversely, the poor performance of Brand C may indicate reduced quality herbal formulation, low grade of raw material or less efficient method of extraction that is not able to release or conserve these sensitive bioactive compounds. The similarity in the three various antioxidant assays with different mechanisms (electron transfer in FRAP, hydrogen atom transfer in DPPH and single electron transfer in ABTS) gives solid and multidimensional support to the better antioxidant ability of Brand A.

Elucidation of Anti-inflammatory Mechanisms:

The anti-inflammatory outcomes also give a new insight into this herbal combinations as to how they could exert cardioprotection. Of specific clinical interest is the powerful TNF-a and IL-6 suppression activity of the brand A which are key actors in the inflammatory cascade leading to accelerated atherosclerosis and aggravated post-infarct remodeling. This inhibition

is strongly indicative of the Brand A extract disrupting one of the important upstream signaling pathways, but most probably the NF-kB pathway. NF-kB is a recognized strong activator of LPS through the activation of NF-kB pathway, which results in the translocation of the transcription factor into the nucleus and, consequently, in the transcription of pro-inflammatory genes, such as TNF-a and IL-6. NF-kB activation is an inhibited activity by the polyphenols found in Brand A, especially flavonoids, such as Hawthorn and the curcuminoids found in Turmeric. This they can do by suppression of IkB kinase (IKK), inhibition of degradation of IkB (the inhibitory protein) and hence the sequestration of NF-kB in the cytoplasm. Trying to dampen this central inflammatory switch, the formulation of Brand A will be able to significantly decrease the production of an extensive range of harmful inflammatory mediators, thus providing a more beneficial environment to the survival and functioning of cardiac cells.

Analysis of cardioprotective and anti-apoptotic efficacy:

The H9c2 cell model of oxidative stress is the strongest first-degree indication of cardioprotection. The ability of the Brand A to retain cell viability and significantly lower the rate of apoptosis in the presence of a powerful oxidant such as H_2O_2 is a testimonial to an effective protective action. This is not just a correlative observation but a causal one: pre-treatment with the extract is prophylactic against the cardiomyoblasts. The anti-apoptotic activity, which is demonstrated by the sharp decrease in the number of Annexin V-positive cells, is a crucial process. Apoptosis induced by H_2O_2 has been known to be carried out by the intrinsic (mitochondrial) route, which entails depolarization of the mitochondrial membrane, release of cytochrome c and activation of caspase-3. This abundance of antioxidants in Brand A is probably to be a direct counter to the H_2O_2 and other ROS in the cell and later prevent the initial oxidative inducement of mitochondrial dysfunction. Moreover, a number of its constituent phytochemicals, including curcumin and particular Hawthorn flavonoid have been reported to directly activate anti-apoptotic proteins like Bcl-2 and reduce pro-apoptotic proteins like Bax, in addition to inhibiting the executioner caspase-3. Thus, the formulation of Brand A can be said to operate in a dual way, in the form of a primary antioxidant defense that suppresses the ROS, and the secondary direct effect on the apoptotic cascade of signal transduction.

Overall Comparative Analysis and Limitations of the Study:

When the findings of the entire assays are combined, a definite and undisputable ranking appears. Of the two formulations, brand A is the most holistically viable, exhibiting excellence in phytochemical content, antioxidant activity, anti-inflammatory effect, and direct cytoprotection activities against the effects of oxidative stresses causing apoptosis. Brand B may be defined as a product of moderate effect whereas Brand C seems to be the least effective in the conditions of the experiment. This extreme inconsistency highlights one of the most pressing problems in the nutraceutical sector the absence of a standardization that is enforced. Even a consumer who bought an herbal heart supplement would experience and result in health outcomes which are radically different in relation to the particular brand purchased. It is important to note though the weakness of this work. To begin with, they are in vitro and it does not take into consideration the complicated pharmacokinetics of absorption, distribution, metabolism, and excretion in vivo. Presumably, the efficacy of the compounds in a living organism is subject to change due to its bioavailability of the compounds in the extracts. Secondly, although we have compared activity with total phenolic and flavonoid content, we are not yet able to identify the individual active compounds, and

the synergistic or antagonistic interactions of these compounds in the complex mixture. Thirdly, the single aqueous extraction procedure, although applicable to some traditional preparations, might not be sufficiently effective to extract lipophilic active constituents (e.g. some terpenes) that would be extracted with alcohol or other solvents. Bioassay-guided fractionation should be used in future studies to identify the main active principles.

5. Conclusion and Future Perspectives:

Moreover, this intensive in vitro study serves as solid scientific evidence that there is substantial difference in cardioprotective potential in commercial herbal cocktail supplements. It manages to establish that Brand A formulation has a better phytochemical profile, which in this case is reflected in the strongest antioxidant, anti-inflammatory, and anti-apoptotic activity compared to the other two brands that were analyzed. Its high phenolic and flavonoid content may be seen to be linked with its high biological efficacy through a possible mechanism of its supremacy, which can be seen as plausible. Thus, the main finding of the research is that Brand A possessed a better indication of comprehensive cardioprotective activity in vitro than the Brands B and C. There are two implications of this research. To the scientific and medical community, it offers a validated and multi-assay platform on the initial screening and comparative assessment of the complex herbal preparations. To the consumers and the healthcare practitioners it provides an evidence-based explanation of why some products are more effective than others, so not every supplements are equal and efficacy is an objective measure. This publication advocates the necessity of quality control and scientific substantiation of the nutraceutical industry so that manufacturers invest in the standardization of their products on the basis of biological activity, rather than on the occurrence of the marker compounds.

Future Work:

This research is used as a groundwork to other studies. The second step in this case should be the bioassay-assisted fractionation of the most effective extract (Brand A) with a view of isolating and determining the particular compound or combination of compounds that produces the effects it is observed to have. The results should then be confirmed using an appropriate in vivo model (rat model of isoproterenol-induced myocardial infarction or coronary artery ligation) to establish cardioprotection in a whole-body model. The other pathways that may be investigated further through mechanistic studies include Nrf2/ARE antioxidant gene regulation pathway, PI3K/Akt pro-survival pathway, and cardiac ion channel modulation. Lastly, human trials, or human experiments, would be the last phase in the translation of these encouraging in vitro findings into a real benefit of human health.

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